UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Subject: Glufosinate Ammonium (PC Code 128850). Section 3 Registrations for Transgenic Cotton (ID# - 0F06140), Transgenic Rice (ID# - 0F06210), and Bushberry (ID# - 2E06404). **Summary of Analytical Chemistry and Residue Data.** DB Barcodes: D271110, D271223, D282757, and D283373. Case Numbers: 292945, 293386, and 294699. Submission: S589377, S596735, and S609042. 40 CFR 180.473. MRIDs 45089302, 45089303, 45204404, 45204405, 45204407, 45204408, 45580201,

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Registration Division (7505C)

Aventis requested a Section 3 registration for application of glufosinate ammonium to transgenic rice, transgenic cotton, and cotton and proposed the establishment of the following permanent tolerances for the combined residues of glufosinate ammonium (butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-, monoammnoium salt), 2-acetamido-4-methylphosphinico-butanoic acid, and 3-methylphosphinico-propionic acid expressed as glufosinate ammonium free acid equivalents (see attachment 1 for structures):

rice, grain	1.0 ppm
rice, straw	1.6 ppm
cotton, undelinted seed	3.5 ppm
cotton, gin byproducts	12 ppm

The Interregional Research Project Number 4 (IR-4) requested a Section 3 registration for application of glufosinate ammonium to blueberry and establishment of the following permanent tolerances for the combined residues of glufosinate ammonium (butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-, monoammnoium salt) and 3-methylphosphinico-propionic acid:

•bushberry subgroup	0.10 ppm

•the initial Section F proposed a tolerance in/on blueberry; via personnel communication with Hoyt Jamerson (RD), HED was informed the petitioner revised there proposal to include the entire bushberry crop sub-group

Recommendations

Section 3 registrations were requested by Aventis (transgenic rice and transgenic and nontransgenic cotton) and IR-4 (blueberry). A separate recommendation is written for each. A human health risk assessment will be prepared as a separate document.

Transgenic Rice and Transgenic and Nontransgenic Cotton: Provided the petitioner submits a revised Section F and a revised Section B, the residue chemistry database is sufficient for an unconditional registration and establishment of the following permanent tolerances for the combined residues of glufosinate ammonium (butonoic acid, 2-amino-4-(hydroxymethylphosphinyl) -, monoammonium salt), 2-acetamido-4-methylphosphinico-butanoic acid, and 3-methylphosphinico-propionic acid (all expressed as 2-amino-4-(hydroxymethylphosphinyl) butanoic acid):

rice, grain	1.0 ppm
rice, straw	2.0 ppm
rice, hull	2.0 ppm
cotton, undelinted seed	4.0 ppm
cotton, gin byproducts	15 ppm
egg	0.15 ppm
poultry, meat byproducts	0.60 ppm
poultry, meat	0.15 ppm
poultry, fat	0.15 ppm
milk	0.15 ppm
meat byproducts (cattle, goat, hog, horse, sheep)	6.0 ppm
meat (cattle, goat, hog, horse, sheep)	0.15 ppm
fat (cattle, goat, hog, horse, sheep)	0.40 ppm

Blueberry: Provided the petitioner submits a revised Section F, the residue chemistry database is sufficient for a conditional registration and establishment of the following permanent tolerances for the combined residues of glufosinate ammnoium, 2-acetamido-4-methylphosphinico-butanoic acid, and 3-methylphosphinico-propionic acid (all expressed as 2-amino-4-(hydroxymethylphosphinyl) butanoic acid):

bushberry crop subgroup (13B)	0.15 ppm

The residue chemistry database will be sufficient for unconditional registration provided the petitioner submits a blueberry field trial study conducted in Region 12 (n=1; residue decline data should be included).

Summary of Residue Chemistry Deficiencies

- revised Section B (see 860.1200 Directions for Use; 860.1400 Water, Fish, and Irrigated Crops; and 860.1850/860.1900 Confined/Field Accumulation in Rotational Crops)
- revised Section F (see 860.1500 Crop Field Trials; 860.1520 Processed Food and Feed; and 860.1480 Meat, Milk, Poultry, and Eggs)
- blueberry field trial (see 860.1500 Crop Field Trials)

Background

Technical glufosinate ammonium is a racemic mixture of the D and L enantiomers; only the L enantiomer is herbicidally active. The compound is a non-selective herbicide and acts as an inhibitor of glutamine synthetase which leads to poisoning of the plant by ammonia. Glufosinate ammonium is currently registered for use on both transgenic and nontransgenic crops. The transgenic plants currently registered (canola, sugar beet, corn, soybean) and the transgenic plants requested for registration (rice and cotton) have been engineered to express phosphiothrion-acetyl-transferase (PAT) which enables the plant to metabolize glufosinate ammonium into N-acetyl-glufosinate.

Current registrations include broadcast application to apple, grape, banana, potato (vine desiccant), and tree nut orchards with tolerances for the combined residues of glufosinate ammonium and 3-methylphosphonic propionic acid (both expressed as glufosinate free acid equivalents) ranging from 0.05 - 0.80 ppm (40 CFR 180.473). Glufosinate ammonium is also registered for application to the transgenic varieties of field corn, canola, sugar beet, and soybean with tolerances for the combined residues of glufosinate ammonium, 2-acetamido-4-methylphosphinico butanoic acid, and 3-methylphosphonic propionic acid (all expressed as glufosinate free acid equivalents) ranging from 0.2 - 25.0 ppm. Tolerances are also established for the combined residues of glufosinate ammonium and 3-methylphosphonic propionic acid (both expressed as glufosinate free acid equivalents) as a result of secondary residues in milk, eggs, and the meat, fat and meat byproducts of ruminants and poultry ranging from 0.02 ppm - 0.10 ppm.

The following terms may be used interchangeably (see attachment 1 for structures):

•HOE 039866 = butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-, monoammnoium salt; represents both the D and L enantiomers
•HOE 099730 = N-acetyl-glufosinate = 2-acetamido-4-methylphosphinico butanoic acid; petitioner indicated that HOE 099730 represents only the L-enantiomer; the analytical method used in the magnitude of the residue, processing, feeding, and metabolism studies did not distinguish between the D and L enantiomers
•HOE 061517 = 3-methylphosphonic propionic acid
•glufosinate free acid = 2-amino-4-(hydroxymethylphosphinyl) butanoic acid

860.1200 Directions for Use

The petitioners have proposed application of Liberty® Herbicide (18.19% glufosinate ammonium; soluble concentrate; EPA Reg. No. 264-660) to cotton, transgenic cotton, and transgenic rice and Rely® Herbicide (11.33% glufosinate ammonium; soluble concentrate; EPA Reg. No. 264-652) to bushberries. The Liberty® label indicates a 120-day plant back interval (PBI) for all crops except wheat, barley, buckwheat, millet, oats, rye, sorghum, and triticale where a 70-day PBI is indicated. Both labels prohibit application through irrigation equipment. The Rely® label also prohibits aerial application. The following are summaries of the proposed application scenarios.

Transgenic-Rice: Glufosinate ammonium may be applied as a broadcast spray to transgenic rice from the 1-leaf stage through the mid-tillering stage of development at 0.37-0.44 lbs ai/acre. A maximum of 0.89 lbs ai/acre can be applied per season. Rice is not to be harvested until 70 days after the last application. Surfactant and crop oils are not to be added to the spray solution. A silicon-based anti-foam agent may be added to the spray solution (the formulated product contains an antifoaming agent). Glufosinate ammonium may be applied prior to or after the establishment of a permanent flood. If applied post-flood, the water level should be lowered so that 75% of the foliage is exposed. A minimum spray volume of 10 gallons/acre is indicated for both ground and aerial applications. The label indicates that rice grown for seed may be treated.

The label should include a statement prohibiting the use of rice paddy water for irrigation purposes, as a water source for livestock, and for raising crayfish. **A revised Section B is requested.**

Transgenic-Cotton: Glufosinate ammonium may be applied from planting through the early bloom stage. A maximum of two broadcast over the top applications are permitted at 0.26-0.52 lbs ai/acre (1.04 lbs ai/acre as a broadcast spray). A third application can be made with the spray directed to the lower third of the plant at 0.52 lbs ai/acre. The season maximum application rate is 1.57 lbs ai/acre. A retreatment internval (RTI) of 14 days is specified. Cotton is not to be harvested until 70 days after the last application. A minimum spray volume of 15 gallons/acre and 10 gallons/acre is indicated for ground and aerial applications, respectively. An antifoaming agent and ammonium sulfate may be added to the spray solution (the formulated product contains an antifoaming agent). The petitioner should amend the label indicating that following treatment of cotton, the field may only be rotated to a registered crop (see 860.1850 and 860.1900 Confined/Field Accumulation in Rotational Crops section). **A revised Section B is requested.**

Cotton: Glufosinate ammonium may be applied from planting through the early bloom stage using a hooded sprayer. A maximum of three applications are permitted at 0.26-0.52 lbs ai/acre (season maximum application rate of 1.57 lbs ai/acre). RTI of 14 days is specified. Cotton is not to be harvested until 70 days after the last application. A minimum spray volume of 15 gallons/acre and 10 gallons/acre is indicated for ground and aerial applications, respectively. An antifoaming agent and ammonium sulfate may be added to the spray solution (the formulated product contains an antifoaming agent). The label adequately explains the proposed application scenario for cotton.

Bushberry: Glufosinate ammonium is to be applied as a directed spray (broadcast, banded, or spot treatment) to undesirable vegetation in blueberry fields at up to 1.5 lbs ai/acre. Two applications are permitted per season with a RTI of 28 days (maximum of 3.0 lbs ai/acre/year). Bushberries are not to be harvested until 14 days after the last application. A minimum spray volume of 20 gallons/acre is indicated. A nonionic antifoaming agent may be added to the spray solution (the formulated product contains an antifoaming agent). Cover crops treated with glufosinate ammonium may not be fed to livestock. The label adequately explains the proposed bushberry application scenario.

860.1300 Nature of the Residue - Plants

HED has previously reviewed metabolism studies conducted with nontransgenic (corn, soybean, apple, and lettuce; 8F3607, J. Garbus, 14-Oct-1988 & 8-Aug-1990) and transgenic (corn, soybean, sugar beet, canola, and rice; D227386, M. Rodriguez,7-Mar-1996; D257629, T. Bloem, 9-Jul-1999; 45204405.der.wpd) crops. The transgenic corn, soybean, sugar beet, canola, and rice investigated in the metabolism studies were engineered to express PAT which acetylates glufosinate (herbicidally active) to form N-acetyl-glufosinate (not herbicidally active).

HOE 061517 was the only metabolite identified in the nontransgenic studies (2-40% total radioactive residue (TRR); only soybean leaf, corn stover, and apples were analyzed). The petitioner demonstrated that 40% of the TRR in nontransgenic corn stover was incorporated into protein, starch, cellulose, and lignin. Glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 were the major residues identified in the transgenic crops (40-98% of the TRR). The petitioner demonstrated that for transgenic sugar beet leaves, surface residues are composed of a nearly equal mixture of the D and L enantiomers of glufosinate ammonium while interior residues are composed of almost exclusively D enantiomer of glufosinate ammonium. This indicates that only the L enantiomer of glufosinate ammonium was acetylated to form N-acetyl-glufosinate.

Based on the metabolism and magnitude of the residue studies, the Metabolism Assessment Review Committee (MARC) concluded that the residues of concern in the crops studied, for tolerance expression and risk assessment purposes, are glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 (D282757, T. Bloem, 9-May-2002). HED concludes that the results from the currently available metabolism studies may be translated to blueberry, cotton, transgenic cotton, and transgenic rice.

860.1300 Nature of the Residue - Livestock

HED has previously reviewed lactating goat and laying hen metabolism studies (8F3607, J. Garbus, 14-Oct-1988 & 8-Aug-1990; D211531, M. Rodriguez, 7-Mar-1996). Since more extensive residue identification was performed for the studies reviewed in D211531, only the metabolism studies summarized in D211531 are discussed. The maximum theoretical dietary burdens (MTDB) are as follows: poultry - 3.33 ppm; beef cattle - 15.38 ppm; dairy cattle - 15.22 ppm; and hogs - 8.89 ppm (see 860.1480 Meat, Milk, Poultry, and Eggs)

Lactating goat and laying hen metabolism were dosed with [3,4-^{14}C]-HOE-039866 at 6.5x and 7.4x the MTDB for ruminants and poultry, respectively. TRRs in muscle and fat from both studies were <0.01 ppm and were not further analyzed. Kidney, liver, and milk from the goat study and egg and liver from the hen study were analyzed with 36-90% of the TRR identified as glufosinate ammonium and HOE 064619. N-acetyl-glufosinate was identified as a minor metabolite in both the goat and hen studies (≤5% TRR).

Since the majority of the livestock dietary burden originates from transgenic crops, N-acetyl-glufosinate will be the primary residue in/on treated feed commodities. N-acetyl-glufosinate was found as a minor metabolite in the [3,4-^{14}C]-HOE-039866 livestock metabolism studies indicating that this compound is part of the glufosinate ammonium metabolic pathway for livestock. Based on the metabolism and feeding studies, the MARC determined that the residues of concern in livestock, for tolerance expression and risk assessment purposes, are glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 (D282757, T. Bloem, 9-May-2002).

860.1340 Residue Analytical Methods

Plants: Two analytical methods have been validated by the Analytical Chemistry Branch (ACB) for enforcement of the currently established tolerances: **(1)** nontransgenic - method HRAV-5A was validated by ACB for the determination of glufosinate ammonium and HOE 061517 in/on apple, grape, almond, soybean seed, corn grain, and corn forage (PP # 8F3607, J. Garbus, 14-Sep-1989) and **(2)** transgenic - method BK/01/99 was validated by ACB for determination of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 in/on canola seed and sugar beet root (D258420, T. Bloem, 19-Aug-2000). Both methods involve extraction with water, anion exchange chromatography, derivatization with trimethylorthoacetate, silica gel column clean-up, and quantification via gas chromatography with flame photometric detection (residues expressed as glufosinate free acid equivalents). Method BK/01/99 includes a cation ion exchange column prior to derivatization which fractionates glufosinate ammonium and N-acetyl-glufosinate and allows for speciation of these compounds (both compounds are derivitized to the same compound). This step can be eliminated if separation of these two compounds is unnecessary. The methods do not distinguish between the D and L enantiomers of glufosinate ammonium and N-acetyl-glufosinate.

The MARC has subsequently determined that the residues of concern for the currently registered and proposed transgenic and nontransgenic crops are glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517. HED concludes that HRAV-5A is sufficient for enforcement of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 tolerances in/on the registered/proposed nontransgenic crops for the following reasons (no additional validation data are required): **(1)** the analytical procedures for HRAV-5A and BK/01/99 are essentially identical; **(2)** adequate recovery data for N-acetyl-glufosinate using method BK/01/99 as been attained in/on canola (seed, oil, meal), sugar beet (tops, root, dried pulp, molasses, sugar), corn (grain, forage, fodder, meal, flour, starch, oil), soybeans (seed, hay, meal, hull, oil), rice (grain, straw, bran, hull, polished rice), and cotton (seed gin byproducts, oil, hull, meal); and **(3)** based on the currently available metabolism studies, residues of N-acetyl-glufosinate are unlikely in nontransgenic crops. The analytical methods used in the transgenic cotton and transgenic rice magnitude of the residue and processing studies were similar to method BK/01/95. Since this method has been validated by ACB and adequate validation has been submitted in conjunction with the magnitude of the residue and processing studies, HED concludes that method BK/01/95 is sufficient for enforcement of the rice and cotton tolerances.

The analytical methods used in the field trial and processing studies were similar to the current enforcement methods and are appropriate for data collection purposes.

Livestock: Method HRAV-12 (also known as BK/01/95) has been validated by ACB for determination of glufosinate ammonium and HOE 061517 in/on milk, egg, muscle, and liver (PP# 8F3607, J. Garbus, 26-Oct-1994). Briefly, the method involves extraction with water, protein precipitation with acetone, anion exchange chromatography, derivatization with trimethylorthoacetate, silica gel column clean-up, and quantification via gas chromatography with flame photometric detection (residues expressed as glufosinate free acid equivalents). The method does not distinguish between the D and L enantiomers of glufosinate ammonium.

The MARC has subsequently determined that the tolerance expression for livestock commodities will be for the combined residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517. The petitioner submitted a feeding study in which residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 were monitored in livestock commodities using method BK/03/95 (method was adequately validated; D211531, M. Rodriguez, 7-Mar-1996). Other than including procedures for quantitation of N-acetyl-glufosinate, method BK/03/95 is identical to the current enforcement method. Since BK/03/95 has been validated for determination of N-acetyl-glufosinate in livestock commodities and the analytical procedure is identical to that of current livestock enforcement method, HED concludes that the current enforcement method is sufficient for enforcement of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 livestock tolerances (no additional validation data are necessary).

860.1360 Multiresidue Methods

Glufosinate ammonium, HOE 061517, and N-acetyl-glufosinate were not quantitatively recovered from any of the FDA Multiresidue Testing Protocols. This information has been forwarded to FDA (PP#8F3607, J. Garbus, 14-Aug-1988; PP#5F4578, M. Rodriguez, 10-Oct-1995).

860.1380 Storage Stability

As part of the current petition, blueberry storage stability data were submitted (45580201.der2.wpd). Control blueberry samples were fortified with glufosinate ammonium and HOE 061517 at 1.00 ppm and placed in frozen storage (<-20 C). The samples were extracted after 615 (glufosinate ammonium) and 593 (HOE 061517) days of storage and the resulting extracts were analyzed 78 (glufosinate ammonium) and 71 (HOE 061517) days after extraction (extracts were stored at <-20 C). The percent recoveries for glufosinate ammonium (95, 96, 98) and HOE 061517 (73, 72, 72) were acceptable.

Previously submitted and reviewed frozen storage stability data indicate that glufosinate ammonium and HOE 061517 are stable for 730 days on frozen apples, corn grain, and soybeans (PP#8F3607, J. Garbus, 8-Aug-1990). Additional storage stability data indicated that glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 are stable for 12 months on transgenic soybean seed, forage and hay; for 3 months on soybean oil and meal; for 6 months on transgenic corn grain, fodder and forage; and for 24 months on transgenic sugar beet tops and roots (D211531 and D219069, M. Rodriguez, 7-Mar-1996; D257629, T. Bloem, 9-Jul-1999).

Based on the available storage stability data and since acceptable percent recoveries were attained for fortified samples run concurrent to the treated samples, HED concludes that the storage intervals and conditions for the samples collected as part of the blueberry, rice, and cotton field trial and processing studies are acceptable.

860.1400 Water, Fish, and Irrigated Crops

In support of the rice Section 3 request, the petitioner submitted a study investigating the quantity of residue in/on crops irrigated with rice paddy water treated with glufosinate ammonium (45204404.der.wpd).

Field trial sites in Rosa, LA and Porterville, CA were planted with transgenic rice and glufosinate ammonium was applied twice at 0.45 lbs ai/acre. In Louisiana, both applications were made to soil and the rice field was flooded 1 day after the second application. In California, both applications were made to a flooded rice field. At both sites, five, eight, and sixteen days after the second application, paddy water was used to irrigate test plots planted with grain sorghum (irrigated 71-88 days after planting), radish (irrigated 9-38 days after planting), collard (Louisiana site only; irrigated 49-60 days after planting), and lettuce (California site only; irrigated 27-38 days after planting).

Irrigated crop samples were collected 14 days after the last irrigation and at maturity and analyzed for residues of glufosinate ammonium and HOE 061517. The analytical method did not distinguish between glufosinate ammonium and N-acetyl-glufosinate (no validation data for N-acetyl-glufosinate was submitted with this study). Residues were generally less <0.008 at both the Louisiana and California test sites. However, residue of glufosinate ammonium was found in/on radish top (<0.008 - 0.014 ppm), radish root (<0.008 - 0.024 ppm), and lettuce (<0.008 - 0.009 ppm) and residues of HOE 061517 were found in/on grain sorghum grain (<0.008 - 0.011 ppm), grain sorghum fodder (<0.008 - 0.008 ppm), and radish top (<0.008 - 0.013 ppm). The petitioner has not provided the storage temperature for the crop samples prior to analysis. These data are necessary to validate the crop residue data. Additionally, HED has determined that the residues of concern in drinking water are glufosinate ammonium, HOE 061517, HOE 064619, and N-acetyl-glufosinate. These residues should have been monitored in the irrigated crops.

Despite the missing data, HED can conclude that residues of glufosinate ammonium and HOE 061517 are possible in/on crops irrigated with rice water paddy water treated with glufosinate ammonium. Therefore, the petitioner should include a statement prohibiting the use of treated rice paddy water for irrigation purposes on the proposed label. **A revised Section B is requested.**

860.1480 Meat, Milk, Poultry, and Eggs

Based on the established/recommended tolerances, the following MTDB were calculated: beef cattle - 15.38 ppm (aspirated grain fractions, corn field forage, cannery waste, cotton gin byproducts), dairy cattle - 15.22 ppm (aspirated grain fractions, corn field forage, cannery waste, cotton gin byproducts), poultry - 3.33 ppm (soybean hulls, soybean meal, soybean seed, cotton meal), and hog - 8.89 ppm (aspirated grain fractions, potato culls, cotton meal, soybean seed). Table 1 is a summary of the MTDB calculations.

Two dairy cow and two poultry feeding studies have been submitted, reviewed, and determined to be adequate: **(1)** dairy cows and poultry feed a diet containing a 3:1 mixture of glufosinate ammonium and HOE 061517 (PP#8F3607, J. Garbus, 8-Aug-1990) and **(2)** dairy cows and poultry feed a diet containing a 15:85 mixture of glufosinate ammonium and N-acetyl-glufosinate (D211531, M. Rodriguez, 7-Mar-1996). Two feeding studies were performed on dairy cows and poultry due to the different residues present in transgenic (principally N-acetyl-glufosinate followed by glufosinate ammonium) and non-transgenic crops (principally HOE 061517). The results from the studies are summarized in Tables 2 and 3.

Residues of N-acetyl-glufosinate were not reported in the 3:1 glufosinate ammonium:HOE 061517 dairy cow and poultry feeding studies. Residues of N-acetyl-glufosinate were monitored in the 15:85 glufosinate ammonium:N-acetyl-glufosinate dairy cow and poultry feeding studies. Other than including procedures for quantitation of N-acetyl-glufosinate, the analytical method used in each of the feeding studies were identical. Since the analytical procedures were identical and the livestock metabolism studies indicated that N-acetyl-glufosinate is minor metabolite when livestock are fed glufosinate ammonium, HED concludes that the method used in the 3:1 glufosinate ammonium:HOE 061517 feeding study adequately accounted for N-acetyl-glufosinate.

Table 1: MTDB Calculations

	raw agricultural commodity	tolerance	% DM	% of diet	ppm in diet
beef cattle	aspirated grain fractions	25	85	20	5.88
	corn forage	4.0	40	40	4.00
	cannery waste	4.0	30	35	4.67
	cotton gin byproducts	15	90	5	0.83
	MTDB				15.38
dairy cattle	aspirated grain fractions	25	85	20	5.88
	corn forage	4.0	40	50	5.00
	cannery waste	4.0	30	20	2.67
	cotton gin byproducts	15	90	10	1.67
	MTDB				15.22
poultry	soybean hulls	5.0	90	20	1.11
	soybean meal	2.0	92	40	0.87
	soybean seed	2.0	89	20	0.45
	cotton meal	4.0	89	20	0.90
	MTDB				3.33
hog	aspirated grain fractions	25	85	20	5.88
	potato culls	0.80	20	50	2.00
	cotton meal	4.0	89	15	0.67
	soybean seed	2	89	15	0.34
	MTDB				8.89

Ruminant: Lactating cows were orally dosed for 28 days with either a 15:85 mixture of glufosinate ammonium:N-acetyl-glufosinate (9.1 ppm, 27.3 ppm, and 91.1 ppm) or with a 3:1 mixture of glufosinate ammonium:HOE 061517 (4 ppm, 12 ppm, and 40 ppm). Milk samples were collected daily and at sacrifice samples of muscle, liver, fat, and kidney were collected. Table 2 is a summary of the concentrations of glufosinate ammonium, HOE 061517, and N-acetyl-glufosinate found in the collected tissues and milk.

Based on the results of the ruminant feeding studies and the current MTDB for ruminants, HED concludes that the following tolerance for the combined residue of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 are appropriate: meat (cattle, goat, hog, horse, sheep) - 0.15 ppm; meat byproducts (cattle, goat, hog, horse, sheep) - 6.0 ppm; fat (cattle, goat, hog, horse, sheep) - 0.40 ppm; and milk - 0.15 ppm. **A revised Section F is requested.**

Poultry: Laying hens were orally dosed for 28 days with either a 15:85 mixture of glufosinate ammonium:N-acetyl-glufosinate (0.36 ppm, 1.08 ppm, and 3.6 ppm) or with a 3:1 mixture of glufosinate ammonium:HOE 061517 (4.5 ppm, 13.5 ppm, and 45 ppm). Egg samples were collected daily and at sacrifice samples of muscle, liver, fat, kidney (3:1 study only), and skin (15:85 study only) were collected. Table 3 is a summary of the concentrations of glufosinate ammonium, HOE 061517, and N-acetyl-glufosinate found in the collected tissues and milk.

Based on the results of the poultry feeding studies and the current MTDB for poultry, HED concludes that the following tolerances are appropriate: poultry, meat - 0.15 ppm; poultry, meat byproducts - 0.60 ppm; poultry, fat - 0.15 ppm; and egg - 0.15 ppm. **A revised Section F is requested.**

Table 2: Summary of Dairy Cow Feeding Studies

matrix	ppm (glufosinate free acid equivalents)						
	HOE 039866/ HOE 099730[2]	HOE 061517	HOE 039866/ HOE 099730[2]	HOE 061517	HOE 039866/ HOE 099730[2]	HOE 061517	anticipated residue at 1x MTDB[1]
15:85 glufosinate ammonium:N-acetyl-glufosinate							
	0.6x MTDB		1.8x MTDB		5.9x MTDB		
milk	<0.02	<0.02	<0.02-0.03	<0.02	0.02-0.23	<0.02-0.03	--, 0.09, 0.04
kidney	<0.10	<0.10	<0.10	<0.10	0.11-0.15	<0.10-0.13	--, --, 0.05
liver	<0.10	<0.10	<0.10	<0.10	<0.10	0.25-0.29	--, --, 0.07
muscle	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.02
fat	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.02
3:1 glufosinate ammonium:HOE 061517							
	0.3x MTDB		0.8x MTDB		2.6x MTDB		
milk	<0.02	<0.05	<0.02	<0.05	<0.02	<0.05	--, --, 0.03
kidney	<0.10	0.41	<0.1	2.0	0.13	7.4	1.70, 2.62, 2.90
liver	0.13	1.5	<0.1	4.2	<0.1	10.7	5.43, 5.38, 4.15
muscle	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.04
fat	0.06	<0.05	<0.05	0.08	<0.05	0.16	0.37, 0.16, 0.08

[1] anticipated residue based on results from each of the three dosing groups; anticipated residue was not calculated if residues were <LOQ; if residues were <LOQ at all three dosing levels then an anticipated residue was calculated using the results from the highest dose level

[2] HOE 099730 was not reported in the 3:1 glufosinate ammonium:HOE 061517 feeding study, analytical method would not have distinguished between HOE 039866 and HOE 099730 or the D and L enantiomers of these compounds

Table 2: Summary of Laying Hen Feeding Studies

matrix	ppm (glufosinate free acid equivalents)						
	HOE 039866/ HOE 099730[2]	HOE 061517	HOE 039866/ HOE 099730[2]	HOE 061517	HOE 039866/ HOE 099730[2]	HOE 061517	anticipated residue at 1x MTDB[1]
15:85 glufosinate ammonium:N-acetyl-glufosinate							
	0.1x MTDB		0.3x MTDB		1.1x MTDB		
liver	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	--, --, 0.18
skin	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.09
muscle	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.09
fat	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.09
egg	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.09
3:1 glufosinate ammonium:HOE 061517							
	1.4x MTDB		4.0x MTDB		13.2x MTDB		
liver	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	--, --, 0.02
kidney	<0.05	0.69	0.07	2.00	<0.05	7.80	0.53, 0.52, 0.59
muscle	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.01
fat	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	--, --, 0.01
egg	<0.05	<0.05	<0.05	<0.05	0.07	<0.05	--, --, 0.01

[1] anticipated residue based on results from each of the three dosing groups; anticipated residue was not calculated if residues were <LOQ; if residues were <LOQ at all three dosing levels then an anticipated residue was calculated using the results from the highest dose level

[2] HOE 099730 was not reported in the 3:1 glufosinate ammonium:HOE 061517 feeding study, analytical method would not have distinguished between HOE 039866 and HOE 099730 or the D and L enantiomers of these compounds

860.1500 Crop Field Trials

Detailed reviews concerning the magnitude of the residue data submitted in support of the current petitions can be found in the following reviews: blueberry (45580201.der1.wpd), transgenic cotton (45089303.der.wpd), and transgenic rice (45204406.der.wpd and 45204407.der.wpd).

Bushberry: The petitioner submitted blueberry magnitude of the residue data conducted in Region 1 (n=1), Region 2 (n=2), and Region 5 (n=2). Rely® (soluble concentrate (SC); 11.33% glufosinate ammonium) was applied twice as a spray directed to the soil at 1.50 lbs ai/acre (1x the maximum proposed single and seasonal application rates; RTI - 25-29 days; spray volumes - 20-31 gallon/acre). Blueberries were harvested at maturity 13-15 days after the final application and analyzed for residues of glufosinate ammonium and HOE 061517 (both expressed as glufosinate ammonium free acid equivalents). The method was adequately validated for data collection purposes (storage interval and conditions have also been validated). Combined residues of glufosinate ammonium and HOE 061517 ranged from <0.03 - 0.08 ppm (residues in/on controls were <0.02). The petitioner has not submitted residue decline data.

HED has determined that the tolerance expression for bushberries will be for residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517. Residues of N-acetyl-glufosinate were not monitored in the blueberry magnitude of the residue study. The method used in the blueberry field trials is identical to that used to monitor for residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 in the transgenic cotton and transgenic rice studies summarized below. These studies indicate that glufosinate ammonium and N-acetyl-glufosinate are derivatized to the same compound and quantified together. For this reason and since the metabolism studies indicated that residue of N-acetyl-glufosinate are unlikely in nontransgenic crops, HED is willing to conclude that the submitted blueberry field trial data has adequately accounted for residues of N-acetyl-glufosinate in/on blueberry.

Since residues were generally <LOQ, a 25 % reduction in the number of field trials is appropriate. Tables 3 and 5 of OPPTS suggests the submission of the following field trial data when requesting a bushberry crop subgroup tolerance and residues are <LOQ: Region 1 (n=1), Region 2 (n=2), Region 5 (n=2), and Region 12 (n=1). An additional field trial in Region 12 is needed to fulfill the suggested geographical distribution. **Provided the petitioner agrees to conduct a field trial in Region 12 (n=1; residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 should be monitored; residue decline data should be included), HED concludes that the available field trial data is sufficient to support a 0.15 ppm permanent tolerance for the combined residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 in/on bushberry crop subgroup. A revised section F is requested.**

Transgenic Cotton: The petitioner submitted transgenic cotton magnitude of the residue data conducted in Region 2 (n=1), Region 3 (n=1), Region 4 (n=3), Region 6 (n=2), Region 8 (n=4), and Region 10 (n=3). Each location consisted of a control plot and two treated plots. The 1st treated plot received two over the top broadcast spray applications of glufosinate ammonium at ~0.50 lbs ai/acre (1x and 0.6x the maximum proposed single and seasonal application rates; RTI - 21-53 days). The 2nd treated plot received three applications of glufosinate ammonium at ~0.50 lbs ai/acre with the first and third made using over the top broadcast spray equipment and the second application directed at the bottom third of the plant (1x the maximum proposed single and

seasonal application rates; RTI = 7-28 days). In all cases, glufosinate ammonium was formulated as Liberty™ (water soluble liquid formulation; 18.2% glufosinate ammonium; spray volume - 9-11 gallon/acre). Cotton was harvested by hand (n=6) or mechanically with spindle (n=4) or stripper (n=4) pickers 67-76 days after the last application. Cotton harvested by hand was ginned locally while the mechanically harvested cotton was ginned at Texas A & M University (Bryan, TX). The cottonseed and cotton gin byproduct samples were analyzed for residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 (all expressed as glufosinate ammonium equivalents). The method was adequately validated for data collection purposes (storage interval and conditions have also been validated). Combined residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 in/on cottonseed treated with glufosinate ammonium at ~1.00 lbs ai/acre/season (0.6x) and ~1.50 lbs ai/acre/season (1.0x) ranged from 0.15 - 3.33 and <0.10 - 2.71 ppm, respectively (residues in/on controls <0.05 ppm). Combined residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 in/on cotton gin byproducts treated with glufosinate ammonium at ~1.00 lbs ai/acre/season (0.6x) and ~1.50 lbs ai/acre/season (1.0x) ranged from 0.30 - 7.36 and 0.95 - 11.63 ppm, respectively (residue in/on controls <0.10 ppm; LOQ = 0.10 ppm).

Table 5 of OPPTS suggests the submission of the following field trial data when requesting a cotton tolerance: Region 2 (n=1), Region 4 (n=3), Region 6 (n=1), Region 8 (n=4), and Region 10 (n=3). The geographical distribution of the field trial data is sufficient. HED concludes that the following tolerances are appropriate: cotton, undelinted seed - 4.0 ppm and cotton, gin byproducts - 15 ppm. **A revised Section F is requested.**

Cotton: The petitioner is also requesting hooded spray application to nontransgenic cotton (seasonal total of 1.57 lbs ai/acre). Field trial data depicting only hooded spray applications have not been submitted. Since hooded spray applications are likely to result in residues less than those demonstrated with over the top applications, residue data reflecting only directed applications are unnecessary.

Transgenic Rice: The petitioner submitted transgenic rice magnitude of the residue data conducted in Region 4 (n=9), Region 5 (n=2), Region 6 (n=2), and Region 10 (n=2). Liberty™ (water soluble liquid formulation; 18.2% glufosinate ammonium) was applied twice at 0.45-0.50 lbs ai/acre (1x - 1.1x maximum proposed single application rate) for a seasonal total of 0.88 - 1.02 (1x - 1.2x maximum proposed single application rate (RTI of 12-29 days; spray volume - 10-11 gallon/acre). The applications were either both made to dry ground (n=1), the 1st made to dry ground and the 2nd made to a flooded field (n=7), or both made to a flooded field (n=7). Rice grain and rice straw were harvested at maturity 70-106 days after the final application and analyzed for residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517. The method was adequately validated for data collection purposes (storage interval and conditions have also been validated). Combined residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 in/on rice grain and rice straw ranged from <0.10 - 0.74 ppm and <0.10 - 1.48 ppm, respectively (residues in/on controls were <0.05).

The residue decline data indicated that residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 did not significantly change in/on rice grain and rice straw as the preharvest interval (PHI) increased from 78 to 96 days. A side by side comparison concerning the addition of ammonium sulfate (3.36 lbs ai/acre) to the tank mix was performed at three of the field trial

sites. The resulting residue data indicated that the addition of ammonium sulfate to the spray solution did not effect the concentration of glufosinate ammonium/N-acetyl glufosinate and HOE 061517 in/on rice grain and rice straw. Comparable residues were attained when both applications were made to a flooded field (n=6) or the first application was made to a dry field and the second to a flooded field (n=6). Based on the limited field trial data available, both applications applied to a dry field (n=1) may result in lower residues when compared to the other water management practices tested.

Table 5 of OPPTS suggests the submission of the following field trial data when requesting a rice tolerance: Region 4 (n=11), Region 5 (n=1), Region 6 (n=2), and Region 10 (n=2). Two field trials in Region 4 are necessary to fulfill the suggested geographical distribution. Since the petitioner has conducted an additional field trial in Region 5 and conducted side by side comparison concerning the addition of ammonium sulfate at 3 of the field trials (Regions 4, 5, and 6), HED concludes that additional field trial data are unnecessary. Based on the available field trial data, HED concludes that the following tolerances, for the combined residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 are appropriate: rice, grain 1.0 ppm and rice, straw - 2.0 ppm. **A revised Section F is requested.**

860.1520 Processed Food and Feed

Detailed reviews concerning the processing studies submitted in support of the current petitions can be found in the following reviews: transgenic cotton (45580201.der.wpd) and transgenic rice (45204406.der.wpd)

Cotton: Transgenic cotton was treated at the 4-leaf and early bloom stages with Liberty™ herbicide (water soluble liquid; 18.2% glufosinate ammonium) at ~2.1 lbs ai/acre (4.29 lbs ai/acre total; 4.8x and 2.7x the maximum proposed single and seasonal application rates, respectively). Cotton was mechanically harvested 76 days after the last application and processed into cottonseed, cottonseed meal, cottonseed hull, and cottonseed refined oil. The processed and unprocessed commodities were analyzed for residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 (analytical method and storage interval and conditions were validated). The resulting residue data indicate that the combined residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 reduced in cottonseed refined oil (0.01x) and concentrated in cottonseed hull (1.2x) and cottonseed meal (1.3x).

Based on the cottonseed highest average field trial (HAFT) of 3.24 ppm from the magnitude of the residue study (45089303.der.wpd); the recommended cottonseed tolerance of 4.0 ppm; and the meal (1.3x), hull (1.2x), and refined oil (0.01x) concentration factors, HED concludes that tolerances for cottonseed processed commodities are unnecessary. Tolerances for cottonseed oil, cottonseed meal, and cottonseed hull will be covered by the unprocessed RAC.

Transgenic Rice: Transgenic rice was treated at the 2-4 leaf stage and the 3-4 tiller stage with Liberty™ herbicide (water soluble liquid; 18.2% glufosinate ammonium) at 2.23 lbs ai/acre (4.47 lbs ai/acre total; 5x the maximum proposed single and seasonal application rates). Rice grain was harvested at maturity 78 days after the last application and processed into rice hull, rice bran, and polished rice. The processed and unprocessed commodities were analyzed for residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 (analytical method and storage

interval and conditions were adequately validated). The resulting residue data indicate that the combined residues of glufosinate ammonium/N-acetyl-glufosinate and HOE 061517 reduced in rice bran (0.8x) and concentrated in rice hull (2.8x) and polished rice (1.3x).

Based on the rice grain HAFT of 0.74 ppm from the magnitude of the residue study (45204406.der.wpd) and the rice hull (2.8x) concentration factor, HED concludes that the following tolerances for the combined residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 are appropriate: rice, hulls - 2.0 ppm. **A revised Section F is requested.** Tolerances for rice bran and polished rice will be covered by the unprocessed RAC.

860.1850 and 860.1900 Confined/Field Accumulation in Rotational Crops

A confined rotational crop study has been submitted and reviewed (D211531 and D219069, M. Rodriquez, 7-Mar-1996). Lettuce, radish, and spring wheat were planted 28 and 119 days after the soil was treated with [3,4-^{14}C]-HOE-039866 at 0.9 lbs ai/acre (0.6x and 1.0x the maximum proposed application rate for cotton and rice, respectively; bushberries are not rotated). All samples planted 28 days after treatment were analyzed. HOE 061517 (5-57% TRR) and HOE 064619 (6-10% TRR) were the only compounds identified (a total of 32-64% of the TRR was identified). Except for the wheat commodities, TRRs were ≤0.02 ppm for the samples planted 120 days after treatment (wheat commodities 0.06-0.15 ppm).

A wheat field rotational crop study has also been submitted and reviewed (P. Errico [RD], 6-May-1998). Wheat was planted 73 - 90 days after the soil was treated with glufosinate ammonium at 0.8 lbs ai/acre (0.5x and 0.9x the maximum proposed application rate for cotton and rice, respectively). Wheat forage, hay, straw, and grain were harvested at maturity and analyzed for residues of glufosinate ammonium and HOE 061517 (residues were < LOQ; LOQ = 0.05 ppm).

Based on the confined and field rotational crop studies, the MARC determined that the residues of concern in rotational crops, for tolerance expression and risk assessment purposes, are glufosinate ammonium, HOE 061517, and HOE 064619 (D282757, T. Bloem, 9-May-2002). The Liberty® label indicates a 120-day PBI for all crops except wheat, barley, buckwheat, millet, oats, rye, sorghum, and triticale where a 70-day PBI is indicated. Based on the results from the confined and field rotational studies, HED concludes that the proposed rotational crop restrictions are appropriate for rice. The currently available confined and field rotational crop studies were conducted at 0.5-0.6x the maximum proposed application rate for cotton. As a result, the magnitude of the residues in/on the rotated crops are not representative of that which would be attained following rotation to a cotton field treated with glufosinate ammonium. Therefore, the petitioner should amend the label indicating that following treatment of cotton with glufosinate ammonium, the field may only be rotated to a registered crop. **A revised Section B is requested.**

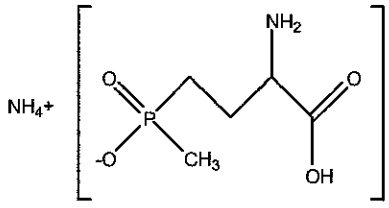
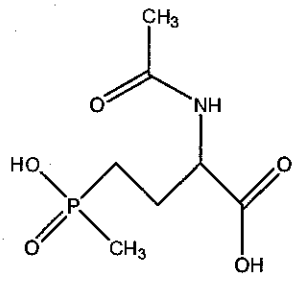
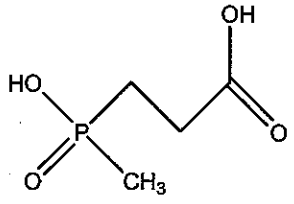
Other Considerations

Codex and Mexico do not have maximum residue limits (MRLs) for residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 in/on the proposed crops or livestock. Canada does not have MRLs for residues of glufosinate ammonium, N-acetyl-glufosinate, and HOE 061517 in/on the proposed crops, poultry commodities, or milk but does have a MRL of 1 ppm for ruminant liver and kidney. The meat byproduct tolerance determined to be appropriate by HED is greater than the Canadian MRL, therefore harmonization is not appropriate.

Attachment 1: Chemical Structures
Attachment 2: 45204405.der.wpd (transgenic rice metabolism study)
Attachment 3: 45580201.der.wpd (storage stability)
Attachment 4: 45204404.der.wpd (water, fish, irrigated crops)
Attachment 5: 45089303.der.wpd (magnitude of the residue, transgenic cotton)
Attachment 6: 45580201.der.wpd (magnitude of the residue, blueberry)
Attachment 7: 45204406.der.wpd (magnitude of the residue, transgenic rice)
Attachment 8: 45204407.der.wpd (magnitude of the residue, transgenic rice)
Attachment 9: 45089302.der.wpd (processed food/feed, transgenic cotton)
Attachment 10: 45204407.der.wpd (processed food/feed, transgenic rice)

cc with all attachments: T. Bloem (RAB1)
RDI: RAB1 Chemist (19-June-2002)
T. Bloem:806R:CM#2:(703)605-0217:7590C

Attachment 1: Chemical Structures

Chemical Name	Chemical Structure
glufosinate ammonium HOE 039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt technical is a racemic mixture of the D and L enantiomers analytical method does not distinguish between the enantiomers	
HOE 099730 IUPAC name - L-2-acetamido-4-methylphosphinico-butanoic acid analytical method can not distinguish between the D and L enantiomers	
HOE 061517 IUPAC name - 3-methylphosphinico-propionic acid	

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45204408. S. Brady (10-Aug-2000). Magnitude of Glufosinate-Ammonium Residues in or on Transgenic Rice Processed Commodities Resulting from Two Applications of Liberty™ Herbicide, USA, 1999. Study Identification BK99R002. Unpublished

Sponsor: Aventis CropScience
Residue Chemistry Department
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Executive Summary

Transgenic rice (Bengal 62) was treated at the 2-4 leaf stage and the 3-4 tiller stage with Liberty™ herbicide (water soluble liquid; 18.2% glufosinate ammonium) at 2.23 lbs ai/acre (4.47 lbs ai/acre total). Rice grain was harvested at maturity 78 days after the last application and processed into rice hull, rice bran, and polished rice. The processed and unprocessed commodities were analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (method was adequately validated). The resulting residue data indicate that the combined residues of HOE 039866/HOE 099730 and HOE 061517 reduced in rice bran (0.84x) and concentrated in rice hull (2.84x) and polished rice (1.29x).

GLP Compliance

The in-life portion of this study was conducted by Coastal Ag Research (East Bernard, TX), the processing facility was the Texas A & M University Food Protein R & D Center (Bryan, TX), and the analytical portion of the study was conducted by Aventis CropScience (Pikeville, NC). Signed and dated Good Laboratory Practices (GLP), quality assurance, and data confidentiality information were provided. The indicated deviations to the study protocol and/or GLP requirements did not effect the conclusions presented in the report.

1. Materials and Methods

1.1. Test Substance

Table 1: Active Ingredient	
Common Name:	glufosinate ammonium
IUPAC Name:	ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate
CAS Name:	butonoic acid, (±)-2-amino-4- (hydroxymethylphosphinyl)-, monoammonium salt
CAS Number:	77182-82-2
Company Name:	HOE 039866
Other Synonyms:	AE F039866, GA

1.2. In-Life Phase

Transgenic rice (Bengal 62) was treated at the 2-4 leaf stage and the 3-4 tiller stage with Liberty™ herbicide (water soluble liquid; 18.2% glufosinate ammonium) at 2.23 lbs ai/acre (4.47 lbs ai/acre total; East Bernard, TX; Region 6). Rice grain was harvested at maturity 78 days after the last application.

The transgenic rice contains phosphiothrion-acetyl-transferase (PAT) which enables the plant to metabolize glufosinate ammonium into a N-acetyl glufosinate (HOE 099730; not herbicidally active).

1.3 Processing Information

The rice grain was oven dried to a moisture content of 12.2-13.1%. The dried rice grain was dehulled and the resulting brown rice was decorticated in an abrasion mill. After decorticating the sample was classified as white milled rice and bran using a 14 TMS screen. Hull accounted for approximately 18% of the unprocessed rice and bran accounted for 11-17% of the brown rice.

1.4 Post Harvest/Collection Storage

The harvested rice grain was placed in frozen storage within 3 hours of collection (temperature was not provided). One day after harvest, the grain sample was shipped via freezer truck to the Food Protein R & D Center of Texas A & M University (Bryan, TX; transport took 25 days). Upon arrival, the grain sample was placed in frozen storage (≤-12 C). The rice grain was processed into polished rice, hulls, and bran within 29 days of harvest. The processed commodities were frozen immediately after collection (≤-12 C). The processed and unprocessed samples were shipped via overnight delivery to Aventis CropScience (Pikeville, NC). Upon arrival at the analytical facility the samples were placed in frozen storage (temperature was not provided).

The rice grain, polished rice, rice bran, and rice hulls samples were extracted within 266, 253, 266, and 265 days of collection, respectively. The extracts were analyzed for residue of HOE 039866/HOE 099730 and HOE 061517 within 9 days of extraction (storage temperature was not provided).

Previously submitted and reviewed frozen storage stability data indicate that HOE 039866 and HOE 061517 are stable for 730 days on frozen apples, corn grain, and soybeans (PP#8F3607, J. Garbus, 8-Aug-1990) and 693 days on blueberries (homogenate stored for 615 days; extract stored for 78 days; 45580201.002.wpd). Additional storage stability data indicated that HOE 039866, HOE 061517, and HOE 099730 are stable for 12 months on transgenic soybean seed, forage, and hay; for 3 months on soybean oil and meal; for 6 months on transgenic corn grain, fodder, and forage; and for 24 months on transgenic sugar beet tops and roots (D211531 and D219069, M. Rodriguez, 7-Mar-1996; D257629, T. Bloem, 9-Jul-1999).

Based on the variety of crops tested (fruit, cereal grain, legume vegetable, root vegetable, and canola), HED concludes that the available storage stability data are sufficient to validate the storage intervals for the rice grain, polished rice, rice hulls, and rice bran samples collected as part of the current study. Since acceptable percent recoveries were attained for fortified control samples run concurrent to the treated samples, HED concludes that the storage intervals for the extracts are acceptable.

Table 2: Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (C)	Duration (days)
unprocessed rice grain	RAC	frozen, temperature not provided	266
	extract	temperature not provided	2
polished rice	RAC	frozen, temperature not provided	253
	extract	temperature not provided	1
rice hull	RAC	frozen, temperature not provided	266
	extract	temperature not provided	9
rice bran	RAC	frozen, temperature not provided	265
	extract	temperature not provided	2

1.5. Analytical Methods

The processed and unprocessed rice samples were analyzed for residues of HOE 039866/HOE 099730 and HOE-061517 using method BK/01/99. The method involves extraction with water, anion exchange, derivatization, silica gel column clean-up, and quantitation via gas chromatography with flame photometric detection. The dervatization step calls for the use of trimethylorthoacetate which esterifies the phosphinic and carboxylic acid function group of glufosinate, HOE 061517, and HOE 099730 and also acetylates the basic amino group of glufosinate. The analytical method does not distinguish between HOE 039866 and HOE 099730.

Based on the percent recoveries from the fortified control samples, HED concludes that the limit of quantitaiton (LOQ) for all analytes in/on unprocessed rice, rice bran, and polished rice is 0.05 ppm. Despite the low recovery of HOE 061517 in polished rice fortified at 0.05 ppm, HED concluded that a LOQ of 0.05 ppm was appropriate based on the low standard deviation. However a correction factor of 0.4 will be applied to polished rice HOE 061517 residues. Acceptable percent recoveries for HOE 061517 and HOE 099730 were attained in/on rice hull fortified at 0.05 ppm. However, acceptable

recoveries of HOE 039866 were only attained in/on rice hull fortified at 1.00 ppm. Therefore, the LOQs for HOE 039866/HOE 099730 and HOE 061517 in/on rice hull are 1.00 ppm and 0.05 ppm, respectively.

Table 3: Percent Recovery from Fortified Control Samples.

Matrix	Fortification Level (ppm)	% Recovery			Mean % Recovery ± Std Dev		
		HOE 039866	HOE 061517	HOE 099730	HOE 039866	HOE 061517	HOE 099730
rice grain	0.05	78	68, 71	80	--	70 ± 2	--
	1.00	76	92, 78	97	--	85 ± 10	--
rice hull	0.05	168, 166, 99	100, 99, 85	117	144 ± 39	95 ± 8	--
	1.00	90	95, 87	101	--	91 ± 6	--
	0.1	--	85	--	--	--	--
polished rice	0.05	70	50, 60, 56, 61	89, 80, 92	--	57 ± 5	87 ± 6
	1.00	87	83, 81, 82, 84	104, 89, 96	--	82 ± 1	96 ± 8
	0.1	--	--	100	--	--	--
rice bran	0.05	--	89	89	--	--	--
	1.00	85	89		--	--	--

2. Results

Table 4: Residues of Imazethapyr, CL 288511, and CL 182704 in/on Rice Grain and Rice Grain Processed Commodities.

Commodity	Residue Levels (ppm)[1]			concentration/reduction factors[2]		
	HOE 039866/ HOE 099730	HOE 061517	total	HOE 039866/ HOE 099730	HOE 061517	total
rice grain	0.29	0.41	0.70	--	--	--
rice hull	<LOQ[3]	0.99	1.99	3.45	2.41	2.84
rice bran	0.36	0.24	0.60	1.24	0.59	0.86
polished rice	0.71[4]	0.19	0.90	2.46	0.46	1.29

1 ppm glufosinate ammonium equivalents; residue in/on controls were non-detect (no peak was present)
2 residue in processed commodity ÷ residue in unprocessed commodity; 1/2 LOQ assumed for residues <LOQ
3 LOQ = 1.00 ppm
4 0.4 correction factor applied

3. Discussion

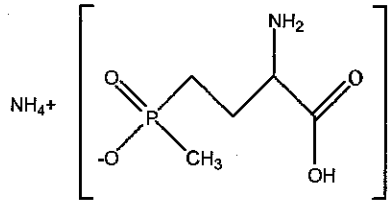
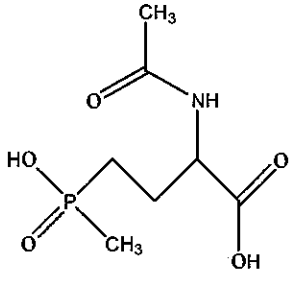
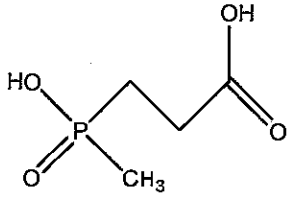
Transgenic rice (Bengal 62) was treated at the 2-4 leaf stage and the 3-4 tiller stage with Liberty™ herbicide (water soluble liquid; 18.2% glufosinate ammonium) at 2.23 lbs ai/acre (4.47 lbs ai/acre total). Rice grain was harvested at maturity 78 days after the last application and processed into rice hull, rice bran, and polished rice. The processed and unprocessed commodities were analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (method was adequately validated). The resulting residue data indicate that the combined residues of HOE 039866/HOE 099730 and HOE 061517 reduced in rice bran (0.84x) and concentrated in rice hull (2.84x) and polished rice (1.29x).

4. Deficiencies

No data gaps were identified in this study.

5. Structures

Table 7: Chemical Name and Structures

Chemical Name	Chemical Structure
glufosinate ammonium HOE 039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt technical is a racemic mixture of the D and L enantiomers analytical method does not distinguish between the enantiomers	
HOE 099730 IUPAC name - L-2-acetamido-4-methylphosphinico-butanoic acid analytical method can not distinguish between the D and L enantiomers	
HOE 061517 IUPAC name - 3-methylphosphinico-propionic acid	

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45580201. F. Salzman (7-Jan-2002). Glufosinate-Ammonium: Magnitude of the Residue on Blueberry. Study Number 05291. Unpublished

Sponsor: IR-4 Project
Rutgers, The State University of New Jersey
681 U.S. Highway 1 South
North Brunswick, NJ 08902-3390

Executive Summary

The petitioner submitted blueberry magnitude of the residue data conducted in Region 1 (n=1), Region 2 (n=2), and Region 5 (n=2). Rely® (soluble concentrate (SC); 11.33% glufosinate ammonium) was applied twice as a spray directed to the soil surface at 1.50 lbs ai/acre (total application rate of 3.0 lbs ai/acre, retreatment interval (RTI) of 25-29 days; spray volume - 20-31 gallon/acre). Blueberries were harvested at maturity 13-15 days after the final application and analyzed for residues of HOE 039866 and HOE 061517 (both analytes expressed as glufosinate ammonium free acid equivalents; the method was adequately validated). Residues of HOE 039866 and HOE 061517 were <0.02 - 0.07 ppm and <0.01 - 0.01 ppm, respectively (residues in/on controls <0.02). Combined residues of HOE 039866 and HOE 061517 ranged from <0.03 - 0.085 ppm. The petitioner has not submitted blueberry residue decline data.

GLP Compliance

The in-life portion of this study was conducted by Rutgers Research and Development, University of New Hampshire, North Carolina State University, and Michigan State University and the analytical portion of the study was conducted by USDA-ARS Environmental Chemistry laboratory (Beltsville, MD). Signed and dated Good Laboratory Practices (GLP), quality assurance, and data confidentiality information were provided. The indicated deviations to the study protocol and GLP requirements did not effect the conclusions presented in the report.

1. Materials and Methods

1.1. Test Substance

Table 1: Active Ingredient	
Common Name:	glufosinate ammonium
IUPAC Name:	ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate
CAS Name:	butonoic acid, (±)-2-amino-4- (hydroxymethylphosphinyl)-, monoammonium salt
CAS Number:	77182-82-2
Company Name:	HOE 039866
Other Synonyms:	AE F039866, GA

1.2. Trial Locations

Table 2: Blueberry Field Trial Locations[1]														
blueberry	Growing Region													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Submitted	1	2	-	-	2	-	-	-	-	-	-	-	-	5
Requested[2]	1	3	-	-	3	-	-	-	-	-	-	1	-	8
	1	2	-	-	2	-	-	-	-	-	-	1	-	6

[1] specific trial information, including state, crop varieties, application method and application rate and timing, can be found in Table 5

[2] second entry is for situation where a 25% reduction in the number of filed trials is possible due to residues <limit of quantitation (LOQ)

1.3. Post-harvest Procedures

The blueberry samples were placed in frozen storage within 2.25 hours of harvest (≤-14 C). The samples were shipped frozen 22 days after collection via ACDS freezer truck or personnel vehicle to the USDA-ARS Environmental Chemistry Laboratory (Beltsville, MD). Upon arrival at the analytical facility, the samples were homogenized and placed in frozen storage (<-20 C). The samples were extracted within 649 days of collection and the extract was analyzed for residue of HOE 039866 and HOE 061517 within 90 days of extraction. Storage stability data has been submitted which indicates that residues of HOE 039866 and HOE 061517 are stable in/on blueberry when stored

frozen for 593 and are stable in extracts when stored frozen for 78 days (45580201.der2.wpd). Previously submitted and reviewed frozen storage stability data indicate that HOE 039866 and HOE 061517 are stable for 730 days on frozen apples, corn grain, and soybeans (PP#8F3607, J. Garbus, 8-Aug-1990).

Based on the available storage stability data, and since acceptable percent recoveries were attained for fortified samples run concurrent to the treated samples, HED concludes that the storage intervals and conditions for the raw agricultural commodities (RACs) and extracts collected as part of the current study are acceptable.

Table 3: Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (C)	Duration (days)
blueberry	RAC	≤-14	649
	Extract	<-20	92

1.4. Analytical Methods

The blueberry samples were analyzed for residues of HOE 039866 and HOE-061517 using a modified version of Hoechst-Roussel-Agri-Vet Company Method HRAV-5A. The method involves extraction with water, anion exchange, derivatization, silica gel column clean-up, and quantification via gas chromatography with flame photometric detection (residues expressed as glufosinate free acid equivalents). The dervatization step calls for the use of trimethylorthoacetate which esterifies the phosphinic and carboxylic acid function group of glufosinate and HOE 061517 and also acetylates the basic amino group of glufosinate. The petitioner reported a LOQ of 0.05 ppm and a limit of detection (LOD) of 0.02 ppm for glufosinate ammonium and a LOQ of 0.03 and a LOD of 0.01 for HOE 061517.

Residues of HOE 039866 were 0.038, 0.063 (n=2), and 0.069 ppm in/on control samples during the initial method validation procedures. Residues of HOE 039866 were <0.02 ppm in/on the remaining control samples (n=16). Residues of HOE 061517 were <0.01 ppm in/on all of the control samples (n=20). The method has been adequately validated for data collection purposes.

Table 4: Percent Recovery from Fortified Control Samples.

Crop Matrix	Fortification Level (ppm)	% Recovery		Mean % Recovery ± SD	
		HOE-039866	HOE-061517	HOE-039866	HOE-061517
blueberry (validation)	0.05[1]	120,132, 132	82-120, 132 (n=6)	128 ± 7	102 ± 33
	0.05	90, 94, 114	--	99 ± 11	--
	1.00[1]	117, 124, 131	58, 61, 75-91 (n=6)	124 ± 7	80 ± 11
	1.00	98, 107, 114	--	106 ± 8	--
blueberry (concurrent)	0.05	100-114, 126 (n=8)	68, 76-92 (n=8)	108 ± 10	79 ± 7
	1.00	74-105, 122, 121, 136 (n=8)	71-86 (n=8)	107 ± 19	79 ± 6

[1] control sample in the initial validation run had HOE 039866 residues of 0.038, 0.063, and 0.069 ppm

2. Results

Table 5: Crop and Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Formulation	App. Method	App. Rate (lbs ai/A)		RTI (days)	App. Timing	Tank Mix Adjuvants	Harvest Method	Plant Part	PHI[4] (days)	Residue (ppm)[5]		
				single	total							HOE 039866	HOE 061517	total
Dunham, NH Region 1	Nelson and Blue Ray	Rely®[1]	directed spray[2]	2 x ~1.5	2.89	29	post-petal fall and 2 weeks preharvest	none	hand	mature berry	14	0.022, 0.013	nd, nd	<0.032, <0.023
Bridgeton, NJ Region 2	Blue Ray and Duke	Rely®[1]	directed spray[2]	2 x ~1.5	3.00	25	light fruit set and fruit set	none	hand	mature berry	13	0.041, 0.072	nd, 0.013	<0.051, 0.085
Castle Hayne, NC[3] Region 2	Croatan	Rely®[1]	directed spray[2]	2 x ~1.5	3.01	29	post bloom and fruiting	none	hand	mature berry	15	nd, nd	nd, nd	<0.03, <0.03
Castle Hayne, NC[3] Region 2	Croatan	Rely®[1]	directed spray[2]	2 x ~1.5	3.01	26	late bloom and green fruit	none	hand	mature berry	15	nd, nd	0.013, 0.013	<0.033, <0.033
East Lansing, MI Region 5	Jersey	Rely®[1]	directed spray[2]	2 x ~1.5	3.02	28	green fruit and green fruit	none	hand	mature berry	14	nd, nd	nd, nd	<0.03, <0.03
Onondaga, MI Region 5	Blue	Rely®[1]	directed spray[2]	2 x ~1.5	3.04	26	blossom and green fruit	none	hand	mature berry	13	nd, nd	nd, nd	<0.03, <0.03

1 Rely® (264-652) = SC formulation containing 11.33% glufosinate ammonium
2 spray directed to the soil surface (away from the plants); spray volume of 20-31 gallons/acre
3 since the field trials were conducted at the same location at the same time, they will be counted as a single field trial
4 PHI = preharvest interval
5 glufosinate free acid equivalents

Table 6: Summary of Blueberry Residue Data from Crop Field Trials.

Commodity	Total App. Rate (lb ai/A)	PHI (days)	analyte	Residue Levels (ppm)[1]				
				Minimum	Maximum	Mean[2]	Std. Dev.[2]	HAFT[3]
blueberry	~3.0	13-15	HOE 039866	<0.02	0.073	0.019	0.019	--
			HOE 061517	<0.01	0.013	0.007	0.003	--
			combined residue	<0.03	0.085	0.026	0.021	0.068

1 glufosinate free acid equivalents
2 ½ LOD assumed for residues <LOD
3 HAFT = highest average field trial; field trial conducted at Bridgeton, NJ

3. Discussion

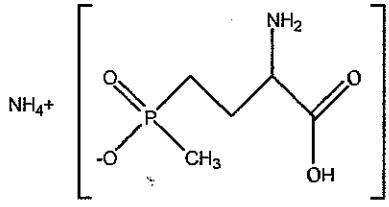
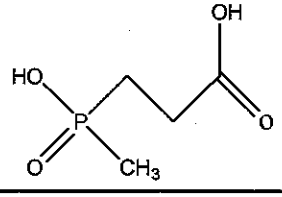
The petitioner submitted blueberry magnitude of the residue data conducted in Region 1 (n=1), Region 2 (n=2), and Region 5 (n=2). Rely® (SC; 11.33% glufosinate ammonium) was applied twice as a spray directed to the soil at 1.50 lbs ai/acre (total application rate of 3.0 lbs ai/acre, RTI of 25-29 days; spray volume - 20-31 gallon/acre). Blueberries were harvested at maturity 13-15 days after the final application and analyzed for residues of HOE 039866 and HOE 061517 (both analytes expressed as glufosinate ammonium free acid equivalents; the method was adequately validated). Residues of HOE 039866 and HOE 061517 were <0.02 - 0.07 ppm and <0.01 - 0.01 ppm, respectively (residues in/on controls <0.02). Combined residues of HOE 039866 and HOE 061517 ranged from <0.03 - 0.085 ppm. The petitioner has not submitted blueberry residue decline data.

4. Deficiencies

The petitioner did not submit blueberry residue decline data.

5. Chemical Structures

Table 7: Chemical Name and Structures

Chemical Name	Chemical Structure
glufosinate ammonium HOE 039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt technical is a racemic mixture of the D and L enantiomers; analytical method does not distinguish between the enantiomers	
HOE 061517 IUPAC name - 3-methylphosphinico-propionic acid	

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45089302. S. Brady (30-Oct-1998). Magnitude of Glufosinate-Ammonium Residues in or on Transgenic Cottonseed Processed Commodities Resulting from Two Applications of Liberty™ Herbicide, USA, 1999. Study Identification BK97R08. Unpublished

Sponsor: AgrEvo USA Company; AgrEvo Research Center
PO Box 538
Pikeville, NC 27863

Executive Summary

Transgenic cotton was treated at the 4-leaf and early bloom stages with Liberty™ herbicide (water soluble liquid; 18.2% glufosinate ammonium) at ~2.1 lbs ai/acre (4.29 lbs ai/acre total). Cotton was mechanically harvested 76 days after the last application and processed into cottonseed, cottonseed meal, cottonseed hull, and cottonseed refined oil. The processed and unprocessed commodities were analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (method was adequately validated). The resulting residue data indicate that the combined residues of HOE 039866/HOE 099730 and HOE 061517 reduced in cottonseed refined oil (0.01x) and concentrated in cottonseed hull (1.18x) and cottonseed meal (1.33x).

GLP Compliance

The in-life portion of this study was conducted by Mid-South Ag Research, Inc (Proctor, AR), the processing facility was the Texas A & M University Food Protein R & D Center (Bryan, TX), and the analytical portion of the study was conducted by EN-CAS Analytical Laboratories (Winston-Salem, NC). Signed and dated Good Laboratory Practices (GLP), quality assurance, and data confidentiality information were provided. The indicated deviations to the study protocol and/or GLP requirements did not effect the conclusions presented in the report.

1. Materials and Methods

1.1. Test Substance

Table 1: Active Ingredient	
Common Name:	glufosinate ammonium
IUPAC Name:	ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate
CAS Name:	butonoic acid, (±)-2-amino-4- (hydroxymethylphosphinyl)-, monoammonium salt
CAS Number:	77182-82-2
Company Name:	HOE 039866
Other Synonyms:	AE F039866, GA

1.2. In-Life Phase

Transgenic cotton (Cot05) was treated at the 4-leaf stage and beginning bloom with Liberty™ herbicide (water soluble liquid; 18.2% glufosinate ammonium) at ~2.1 lbs ai/acre (4.29 lbs ai/acre total; West Memphis, AR; Region 4). Cotton was mechanically harvested 76 days after the last application.

The transgenic cotton contains phosphiothrion-acetyl-transferase (PAT) which enables the plant to metabolize glufosinate ammonium into a N-acetyl glufosinate (HOE 099730; not herbicidally active).

1.3. Processing Information

The cotton was dried and burrs, sticks, and other plant parts were removed. The cotton was then ginned and the resulting seed was mechanically dehulled using a Carver huller. The resulting seed kernal was dried to 12% moisture and flaked. The flaked material was fed into an expander/extruder and steam was injected directly to the product. The exiting material was dried and taken to a stainless steel batch solvent extractor (hexane). After 30 minutes the hexane was drained and the process repeated 2 more times. A portion of the hexane crude oil mixture was removed and passed through a laboratory vacuum evaporator and processed into refined oil.

1.4 Post Harvest/Collection Storage

The harvested cotton was placed in frozen storage within 30 minutes of collection (≤4 C). Six days after harvest, the cotton was shipped via freezer truck to the Texas A & M University Food Protein R & D Center (Bryan, TX; transport took 13 days). Upon arrival the samples were placed in frozen storage (≤-1 C). The cotton was processed within 153 days of harvest into ginned cottonseed, cottonseed meal, and cottonseed refined oil (processed commodities were frozen immediately after collection; ≤-1 C). The samples were shipped frozen via overnight delivery to the AgrEvo Research Center (Pikeville, NC). Upon arrival the samples were placed in frozen storage (temperature was not provided) and were shipped frozen thirteen days later to EN-CAS Laboratories for analysis. Upon arrival at the analytical facility the samples were placed in frozen storage (≤-10 C).

Cottonseed, cottonseed meal, cottonseed hulls, and cottonseed refined oil were extracted within 198, 190 (37 days after collection), 190 (45 days after collection), and 201 (50 days after collection) days of harvest. The resulting extracts were analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 within 7 days of extraction.

Previously submitted and reviewed frozen storage stability data indicate that HOE 039866 and HOE 061517 are stable for 730 days on frozen apples, corn grain, and soybeans (PP#8F3607, J. Garbus, 8-Aug-1990) and 693 days on blueberries (homogenate stored for 615 days; extract stored for 78 days; 45580201.002.wpd). Additional storage stability data indicated that HOE 039866, HOE 061517, and HOE 099730 are stable for 12 months on transgenic soybean seed, forage, and hay; for 3 months on soybean oil and meal; for 6 months on transgenic corn grain, fodder, and forage; and for 24 months on transgenic sugar beet tops and roots (D211531 and D219069, M. Rodriguez, 7-Mar-1996; D257629, T. Bloem, 9-Jul-1999).

Based on the variety of crops tested (fruit, cereal grain, legume vegetable, root vegetable, and canola), HED concludes that the available storage stability data is sufficient to validate the storage intervals for the cottonseed, cottonseed meal, cottonseed hulls, and cottonseed refined oil samples collected as part of the current study. The storage temperature for the sample extracts was not provided. Since the concurrent percent recovery data were acceptable, the storage conditions and intervals for the extracts are acceptable.

Table 2: Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (C)	Duration (days)
cottonseed	RAC	frozen, temperature not provided	198
	extract	temperature not provided	4
cottonseed meal	RAC	frozen, temperature not provided	37days from collection 190 days after harvest
	extract	temperature not provided	7
cottonseed hull	RAC	frozen, temperature not provided	45 days from collection 190 days after harvest
	extract	temperature not provided	7
rice bran	RAC	frozen, temperature not provided	50 days from collection 201 days after harvest
	extract	temperature not provided	2

1.5. Analytical Methods

The cotton samples were analyzed for residues of HOE 039866/HOE 099730 and HOE-061517 using method BK/05/95. The seed, hull, and meal samples were extracted with water, passed through an anion exchange column, derivatized with trimethylorthoacetate, passed through a silica gel column, and quantified via gas chromatography with flame photometric detection (residues expressed as glufosinate ammonium free acid equivalents). Refined oil was refluxed with trimethylorthoacetate (4.5 hours) and extracted with toluene. The toluene extract was passed through a silica gel column and quantified via gas chromatography with flame photometric detection (residues expressed as

glufosinate ammonium free acid equivalents). Trimethylorthoacetate esterifies the phosphinic and carboxylic acid function group of glufosinate, HOE 061517, and HOE 099730 and also acetylates the basic amino group of glufosinate. The analytical method does not distinguish between HOE 039866 and HOE 099730. The petitioner reported a limit of quantitation (LOQ) of 0.05 ppm for all analytes and matrices. Residues in/on control samples were <LOQ. The method has been adequately validated for data collection purposes.

Table 3: Percent Recovery from Fortified Control Samples.

Matrix	Fortification Level (ppm)	% Recovery			Mean % Recovery ± Std Dev		
		HOE 039866	HOE 061517	HOE 099730	HOE 039866	HOE 061517	HOE 099730
cottonseed	0.05	--	101	117	--	--	--
	5.00	99	91	--	--	--	--
cottonseed meal	0.05	114, 117	69, 81	--	116 ± 2	75 ± 8	--
	0.50	--	89, 91	99, 105		90 ± 1	102 ± 4
	10.00	99	82, 89	116	--	--	--
cottonseed hull	0.05	--	111, 106	83, 72	--	108 ± 4	78 ± 8
	5.00	97, 97	87, 96	--	97 ± 0	92 ± 6	--
cottonseed refined oil	0.05	80	95	--	--	--	--
	5.00	--	92	101	--	--	--

2. Results

Table 4: Residues of Imazethapyr, CL 288511, and CL 182704 in/on Rice Grain and Rice Grain Processed Commodities.

Commodity	Residue Levels (ppm)[1]			concentration/reduction factors[2]		
	HOE 039866/ HOE 099730	HOE 061517	total	HOE 039866/ HOE 099730	HOE 061517	total
cottonseed	0.92	4.14	5.06	--	--	--
	0.80	4.33	5.13	--	--	--
cottonseed meal	0.84	5.16	6	1.05	1.25	1.19
	0.88	5.84	6.72	1.10	1.41	1.33
cottonseed hull	1.19	4.72	5.91	1.49	1.14	1.17
	1.12	4.83	5.95	1.40	1.17	1.18
cottonseed refined oil	<0.05	<0.05	<0.10	0.03	0.01	0.01
	<0.05	<0.05	<0.10	0.03	0.01	0.01

[1] ppm glufosinate ammonium equivalents

[2] residue in processed commodity ÷ residue in unprocessed commodity; 1/2 LOQ assumed for residues <LOQ; lowest residue in unprocessed RAC used in calculation

3. Discussion

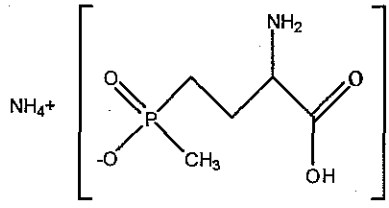
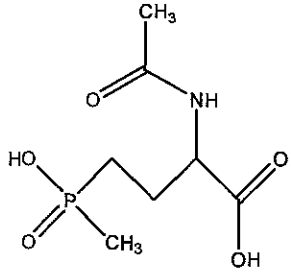
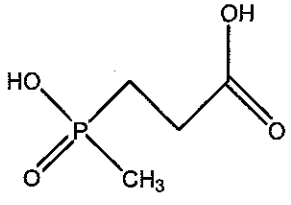
Transgenic cotton was treated at the 4-leaf and early bloom stages with Liberty™ herbicide (water soluble liquid; 18.2% glufosinate ammonium) at ~2.1 lbs ai/acre (4.29 lbs ai/acre total). Cotton was mechanically harvested 76 days after the last application and processed into cottonseed, cottonseed meal, cottonseed hull, and cottonseed refined oil. The processed and unprocessed commodities were analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (method was adequately validated; residue in controls <LOQ). The resulting residue data indicate that the combined residues of HOE 039866/HOE 099730 and HOE 061517 reduced in cottonseed refined oil (0.01x) and concentrated in cottonseed hull (1.18x) and cottonseed meal (1.33x).

4. Deficiencies

No data gaps were identified.

5. Structures

Table 7: Chemical Name and Structures

Chemical Name	Chemical Structure
glufosinate ammonium HOE 039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, *monoammonium* salt technical is a racemic mixture of the D and L enantiomers analytical method does not distinguish between the enantiomers	
HOE 099730 *IUPAC* name - *L*-2-acetamido-4-methylphosphinico-butanoic acid analytical method can not distinquish between the D and *L* enantiomers	
HOE 061517 IUPAC name - 3-methylphosphinico-propionic acid	

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45204406. S. Brady (7-Aug-2000). Magnitude of Glufosinate-Ammonium Residues in or on Transgenic Rice Raw Agricultural Commodities Resulting from Two Applications of Liberty™ Herbicide USA, 1999. Study Identification BK99R001. Unpublished

Sponsor: Aventis CropScience
PO Box 12014
Research Triangle Park, NC 27709

Executive Summary

The petitioner submitted transgenic rice magnitude of the residue data conducted in Region 4 (n=9), Region 5 (n=2), and Region 6 (n=2). Liberty™ (water soluble liquid formulation; 18.2% glufosinate ammonium) was applied twice at 0.45 lbs ai/acre (total application rate of 0.90 lbs ai/acre; retreatment interval (RTI) of 12-29 days; 2-4 leaf stage and 2-4 tiller stage). The applications were either both made to dry ground (n=1), the 1st made to dry ground and the 2nd made to a flooded field (n=6), or both made to a flooded field (n=6). Rice grain and rice straw were harvested at maturity 70-106 days after the final application and analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (all residues expressed as glufosinate ammonium; method was adequately validated). Combined residues of HOE 039866/HOE 099730 and HOE 061517 in/on rice grain and straw ranged from <0.10 - 0.74 ppm and <0.10 - 1.48 ppm, respectively (residues in/on control samples were <0.05 ppm).

The residue decline data indicated that residues of HOE 039866/HOE 099730 and HOE 061517 did not significantly change in/on rice grain and rice straw as the preharvest interval (PHI) increased from 78 to 96 days. A side by side comparison concerning the addition of ammonium sulfate (3.36 lbs ai/acre) to the tank mix was performed at three of the field trial sites. The resulting residue data indicated that the addition of ammonium sulfate may result in lower residues although these results were not definitive. Comparable residues were attained when both applications were made to a

flooded field (n=6) or the first application was made to a dry field and the second to a flooded field (n=6). Based on the limited field trial data available, both applications applied to a dry rice field (n=1) may result in lower residues when compared to the other water management practices tested.

GLP Compliance

The in-life portion of this study was conducted by several companies and the analytical portion of the study was conducted by AgrEvo Research Center Residue Chemistry Department (Pikeville, NC). Signed and dated Good Laboratory Practices (GLP), quality assurance, and data confidentiality information were provided. The indicated deviations to the study protocol and/or GLP requirements did not effect the conclusions presented in the report.

1. Materials and Methods

1.1. Test Substance

Table 1: Active Ingredient	
Common Name:	glufosinate ammonium
IUPAC Name:	ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate
CAS Name:	butonoic acid, (±)-2-amino-4- (hydroxymethylphosphinyl)-, monoammonium salt
CAS Number:	77182-82-2
Company Name:	HOE 039866
Other Synonyms:	AE F039866, GA

1.2. Trial Locations

Table 2: Transgenic Rice Field Trial Locations[1]														
transgenic rice	Growing Region													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Submitted	-	-	-	9	2	2	-	-	-	-	-	-	-	13
Requested[2]	-	-	-	11	1	2	-	-	-	2	-	-	-	16
	-	-	-	7	1	2	-	-	-	2	-	-	-	12

[1] specific trial information, including state, crop varieties, application method and application rate and timing, can be found in Table 5

[2] second entry is for situation where a 25% reduction in the number of filed trials is possible due to residues <limit of quantitation (LOQ)

1.3. Post-harvest Procedures

The rice grain and straw samples were placed in frozen storage within 2.25 hours of harvest (temperature was not provided). The samples were shipped frozen 22 days after collection via ACDS freezer truck or personnel vehicle to the AgrEvo Research Center (Pikeville, NC). Upon arrival at the analytical facility, the samples were homogenized and placed in frozen storage (temperature was not provided). The rice grain and rice straw samples were extracted within 272 and 281 days, respectively, and the extracts were analyzed for residue of HOE 039866/HOE 099730 and HOE-061517 within 37 days of extraction (storage temperature was not provided).

Previously submitted and reviewed frozen storage stability data indicate that HOE 039866 and HOE 061517 are stable for 730 days on frozen apples, corn grain, and soybeans (PP#8F3607, J. Garbus, 8-Aug-1990) and 693 days on blueberries (homogenate stored for 615 days; extract stored for 78 days; 45580201.002.wpd). Additional storage stability data indicated that HOE 039866/HOE 099730 and HOE 061517 are stable for 12 months on transgenic soybean seed, forage and hay; for 3 months on soybean oil and meal; for 6 months on transgenic corn grain, fodder and forage; and for 24 months on transgenic sugar beet tops and roots (D211531 and D219069, M. Rodriguez, 7-Mar-1996; D257629, T. Bloem, 9-Jul-1999).

Based on the variety of crops tested (fruit, cereal grain, legume vegetable, root vegetable, and canola), HED concludes that these data are sufficient to validate the storage intervals for the rice straw and rice grain raw agricultural commodities (RACs) collected as part of the current study. Since the percent recoveries for fortified control samples run concurrent to the treated samples were acceptable, the storage conditions and intervals for the extracts are acceptable.

Table 3: Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (C)	Duration (days)
rice grain	RAC	stored frozen; temperature was not provided	272
	extract	temperature was not provided	29
rice straw	RAC	stored frozen; temperature was not provided	281
	extract	temperature was not provided	37

1.4. Analytical Methods

The rice grain and straw samples were analyzed for residues of HOE 039866/HOE 099730 and HOE-061517 using method BK/01/99. The method involves extraction with water, anion exchange, derivatization, silica gel column clean-up, and quantitation via gas chromatography with flame photometric detection (residue expressed as glufosinate ammonium equivalents). The dervatization step calls for the use of trimethylorthoacetate which esterifies the phosphinic and carboxylic acid function group of glufosinate, HOE 061517, and HOE 099730 and also acetylates the basic amino group of glufosinate. The analytical method does not distinguish between HOE 039866 and HOE 099730. The petitioner reported a LOQ of 0.05 ppm for each analyte (limit of detection was not reported). Residues in/on the control samples were <0.05 ppm. The method has been adequately validated for data collection purposes.

Table 4: Percent Recovery from Fortified Control Samples.

Crop Matrix	Fortification Level (ppm)	analyte	% Recovery	Mean % Recovery ± Std Dev
rice grain	0.05	HOE 039866	71-87 (n=3)	80 ± 8
		HOE 061517	63-102 (n=9)	82 ± 11
		HOE 099730	88-102, 124, 127 (n=6)	104 ± 17
	0.10	HOE 039866	81	--
		HOE 061517	73, 75	74 ± 1
		HOE 099730	89	--
	0.40	HOE 039866	82, 85	83 ± 2
		HOE 061517	83, 94	89 ± 7
		HOE 099730	--	--
	1.00	HOE 039866	--	--
		HOE 061517	77-97 (n=3)	83 ± 11
		HOE 099730	84, 88, 128	100 ± 24
rice straw	0.05	HOE 039866	73-79 (n=3)	76 ± 3
		HOE 061517	63, 65, 74-91 (n=7)	76 ± 10
		HOE 099730	80-92 (n=4)	86 ± 6
	1.00	HOE 039866	102	--
		HOE 061517	71-88 (n=5)	77 ± 7
		HOE 099730	80-90 (n=3)	85 ± 5

2. Results

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Formulation	App. Method[1]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[8]		
												HOE 039866/ HOE 099730	HOE 061517	total
Newport, AR Region 4[1]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.90	23	none	grain	thresher	96	0.19, 0.20	<0.05, <0.05	<0.24, <0.25
				0.45	3 tiller				straw	hand sickle	96	0.18, 0.22	0.13, 0.15	0.31, 0.37
Oil Trough, AR Region 4[1]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.91	22	none	grain	thresher	94	<0.05, <0.05	<0.05, <0.05	<0.10, <0.10
				0.46	3 tiller				straw	hand sickle	94	<0.05, <0.05	0.05, <0.05	<0.10, <0.10
Procotor, AR Region 4[2]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.90	26	none	grain	thresher	86	0.09, 0.08	<0.05, <0.05	<0.14, <0.13
				0.45	4 tiller				straw	hand sickle	86	0.05, <0.05	0.06, 0.05	0.11, <0.10
Blackfish Lake, AR Region 4[2]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.90	26	none	grain	thresher	85	0.05, 0.09	<0.05, <0.05	<0.10, <0.14
				0.45	4 tiller				straw	thresher	85	0.12, 0.14	0.11, 0.12	0.23, 0.26
Stuttgart, AR Region 4[3,6]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.93	15	none	grain	combine	95	<0.05, <0.05	<0.05, <0.05	<0.10, <0.10
				0.48	3 tiller				straw	combine	95	<0.05, <0.05	<0.05, 0.07	<0.10, <0.12
Stuttgart, AR Region 4[3,6]	Bengal 62[4]	Liberty™ 5	spray	0.46	3 leaf	0.92	13	none	grain	combine	106	<0.05, <0.05	<0.05, <0.05	<0.10, <0.10
				0.46	2 tiller				straw	combine	106	<0.05, <0.05	<0.05, <0.05	<0.10, <0.10
Washington, LA Region 4[1,7]	Bengal 62[4]	Liberty™ 5	spray	0.45	4 leaf	0.90	23	none	grain	thresher	78	0.46, 0.40	0.09, 0.08	0.55, 0.48
											84	0.33, 0.36	0.09, 0.09	0.42, 0.45
											90	0.33, 0.36	0.06. 0.06	0.39, 0.42
											96	0.32, 0.29	0.06, <0.05	0.38, <0.34
				0.45	3 tiller				straw	thresher	78	0.46, 0.12	0.21, 0.09	0.67, 0.21
											84	0.71, 0.51	0.35, 0.28	1.06, 0.79
											90	0.43, 0.47	0.18, 0.19	0.61, 0.66
											96	0.50, 0.33	0.27, 0.19	0.77, 0.52

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Formulation	App. Method[1]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[8]		
												HOE 039866/ HOE 099730	HOE 061517	total
Washington, LA Region 4[1,7]	Bengal 62[4]	Liberty™ 5	spray	0.45	4 leaf	0.90	23	3.36 lbs ai/acre ammonium sulfate	grain	thresher	78	0.22, 0.21	0.05, 0.07	0.27, 0.28
											84	0.25, 0.24	0.07, 0.06	0.32, 0.30
											90	0.18, 0.14	<0.05, <0.05	<0.23, <0.19
											96	0.19, 0.21	<0.05, <0.05	<0.24, <0.26
				0.45	3 tiller				straw	thresher	78	0.13, 0.51	0.11, 0.22	0.24, 0.73
											84	0.27, 0.20	0.19, 0.15	0.46, 0.35
											90	0.14, 0.14	0.08, 0.11	0.22, 0.25
											96	0.27, 0.28	0.20, 0.20	0.47, 0.48
Washington, LA Region 4[2,7]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.90	23	none	grain	thresher	81	0.17, 0.17	<0.05, <0.05	<0.22, <0.22
				0.45	4 tiller				straw	thresher	81	0.26, 0.25	0.10, 0.12	0.36, 0.37
Washington, LA Region 4[2,7]	Bengal 62[4]	Liberty™ 5	spray	0.43	3 leaf	0.88	18	none	grain	combine	70	0.66, 0.67	0.07, 0.07	0.73, 0.74
				0.45	3 tiller				straw	combine	70	0.47, 0.58	0.09, 0.09	0.56, 0.67
Washington, LA Region 4[1,7]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.91	27	none	grain	combine	70	0.41, 0.42	<0.05, 0.05	<0.46, <0.47
				0.46	4 tiller				straw	combine	70	0.98, 0.76	0.50, 0.36	1.48, 1.12
Greenville, MS Region 4[2]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.91	22	none	grain	combine	77	0.08, 0.07	<0.05, <0.05	<0.13, <0.12
				0.45	4 tiller				straw	combine	77	0.16, 0.14	0.15, 0.15	0.31, 0.29
Shaw, MS Region 4[2]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.90	29	none	grain	thresher	79	<0.05, <0.05	<0.05, <0.05	<0.10, <0.10
				0.45	3 tiller				straw	thresher	79	<0.05, <0.05	0.05, <0.05	<0.10, <0.10

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Formulation	App. Method[1]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[8]		
												HOE 039866/ HOE 099730	HOE 061517	total
Dexter, MO Region 5[2,6]	Bengal 62[4]	Liberty™ 5	spray	0.45	2 leaf	0.90	29	none	grain	thresher	80	0.07, 0.07	<0.05, <0.05	<0.12, <0.12
											84	0.10, 0.08	0.06 <0.05	0.16, <0.13
											88	0.08, 0.08	<0.05, 0.05	<0.13, <0.13
											92	0.05, 0.09	<0.05, <0.05	<0.10, <0.14
											96	0.05, 0.05	<0.05, <0.05	<0.10, <0.10
				0.45	4 tiller				straw	thresher	80	0.19, 0.11	0.16, 0.11	0.35, 0.22
											84	0.11, 0.09	0.08, 0.10	0.19, 0.19
											88	0.07, 0.08	0.07, 0.09	0.14, 0.17
											92	0.09, 0.09	0.09, 0.08	0.18, 0.17
											96	0.06, 0.05	0.06, <0.05	0.12, <0.10
Dexter, MO Region 5[2,6]	Bengal 62[4]	Liberty™ 5	spray	0.45	2 leaf	0.90	29	3.36 lbs ai/acre ammonium sulfate	grain	thresher	80	<0.05, 0.06	<0.05, <0.05	<0.10, <0.11
											84	0.06, 0.06	<0.05, <0.05	<0.11, <0.11
											88	0.08, <0.05	<0.05, <0.05	<0.13, <0.10
											92	0.07, <0.05	<0.05, <0.05	<0.12, <0.10
											96	0.06, <0.05	0.05, <0.05	0.11, <0.10
				0.45	4 tiller				straw	thresher	80	0.06, 0.09	0.08, 0.14	0.14, 0.23
											84	<0.05, 0.06	<0.05, 0.06	<0.10, 0.12
											88	0.07, 0.10	0.07, 0.08	0.14, 0.18
											92	<0.05, <0.05	0.06, 0.06	<0.11, <0.11
											96	0.07, <0.05	0.11, 0.06	0.18, <0.11
Benton, MO Region 5[1]	Bengal 62[4]	Liberty™ 5	spray	0.46	4 leaf	0.92	17	none	grain	combine	86	0.14, 0.15	<0.05, <0.05	<0.19, <0.20
				0.46	4 tiller				straw	combine	86	0.53, 0.49	0.12, 0.11	0.65, 0.60
East Bernard, TX Region 6[1,6]	Bengal 62[4]	Liberty™ 5	spray	0.45	4 leaf	0.90	12	none	grain	hand sickle	78	0.32, 0.26	0.07, <0.05	0.39, <0.31
				0.45	4 tiller				straw	thresher	78	0.32, 0.35	0.20, 0.19	0.52, 0.54

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Formulation	App. Method[1]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[8]		
												HOE 039866/ HOE 099730	HOE 061517	total
East Bernard, TX Region 6[1,6]	Bengal 62[4]	Liberty™ 5	spray	0.45	4 leaf	0.90	12	3.36 lbs ai/acre ammonium sulfate	grain	hand sickle	78	0.21, 0.19	0.07, 0.08	0.28, 0.27
				0.45	4 tiller				straw	thresher	78	0.25, 0.23	0.17, 0.19	0.42, 0.42
Brookshire, TX Region 6[1]	Bengal 62[4]	Liberty™ 5	spray	0.45	3 leaf	0.90	29	none	grain	thresher	91	0.08, 0.12	<0.05, <0.05	<0.13, <0.17
				0.45	4 tiller				straw	thresher	91	0.18, 0.16	0.19, 0.16	0.37, 0.32

1 field flooded 6-25 days prior to 1st application
2 field flooded 2-28 days after 1st application
3 field flooded 7-16 days after 2nd application
4 Bengal 62 = transgenic rice resistant to glufosinate ammonium; engineered to express phosphiothrion-acetyl-transferase (PAT) which enables the plant to metabolize glufosinate ammonium into a N-acetyl glufosinate (HOE 099730; not herbicidally active)
5 Liberty™ = water soluble liquid formulation; 18.2% glufosinate ammonium
6 since the field trials were conducted at the same location at the same time using the same water management practices, they will be counted as a single field trial
7 4 field trials were conducted at the same location and nearly the same time using 2 different water management practices, therefore these will count as 2 field trials
8 glufosinate ammonium equivalents

Table 6: Summary of Residue Data from Transgenic Rice Field Trials.

Commodity	Total App. Rate (lb ai/A)	PHI (days)	analyte	Residue Levels (ppm)[1]				
				Minimum	Maximum	Mean[1]	Std. Dev.[2]	HAFT[3]
Rice grain	0.90	70-106	HOE 039866/ HOE 099730	<0.05	0.67	0.16	0.15	--
			HOE 061517	<0.05	0.09	0.04	0.02	--
			combined residue	<0.10	0.74	0.20	0.16	0.74[4]
Rice straw	0.90	70-106	HOE 039866/ HOE 099730	<0.05	0.98	0.21	0.21	--
			HOE 061517	<0.05	0.50	0.12	0.09	--
			combined residue	<0.10	1.48	0.34	0.29	1.30[5]

1 glufosinate ammonium equivalents
2 ½ LOQ assumed for residues <LOQ
3 HAFT = highest average field trial
4 3rd Washington, LA field trial in Table 5
5 4th Washington, LA field trial in Table 5

3. Discussion

The petitioner submitted transgenic rice magnitude of the residue data conducted in Region 4 (n=9), Region 5 (n=2), and Region 6 (n=2). Liberty™ (water soluble liquid formulation; 18.2% glufosinate ammonium) was applied twice at 0.45 lbs ai/acre (total application rate of 0.90 lbs ai/acre; RTI of 12-29 days; 2-4 leaf stage and 2-4 tiller stage). The applications were either both made to dry ground (n=1), the 1st made to dry ground and the 2nd made to a flooded field (n=6), or both made to a flooded field (n=6). Rice grain and rice straw were harvested at maturity 70-106 days after the final application and analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (all residues expressed as glufosinate ammonium; method was adequately validated). Combined residues of HOE 039866/HOE 099730 and HOE 061517 in/on rice grain and rice straw ranged from <0.10-0.74 ppm and <0.10-1.48 ppm, respectively (residues in/on control samples were <0.05 ppm).

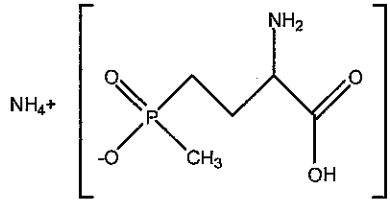
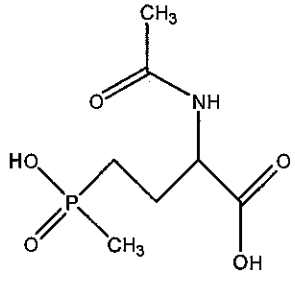
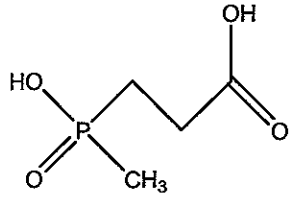
The residue decline data indicated that residues of HOE 039866/HOE 099730 and HOE 061517 did not significantly change in/on rice grain and rice straw as the PHI increased from 78 to 96 days. A side by side comparison concerning the addition of ammonium sulfate (3.36 lbs ai/acre) to the tank mix was performed at three of the field trial sites. The resulting residue data indicated that the addition of ammonium sulfate may result in lower residue although these results were not definitive. Comparable residues were attained when both applications were made to a flooded field (n=6) or the first application was made to a dry field and the second to a flooded field (n=6). Based on the limited field trial data available, both applications applied to a dry rice field (n=1) may result in lower residues when compared to the other water management practices tested.

4. Deficiencies

No data gaps were identified.

5. Chemical Structures

Table 7: Chemical Name and Structures

Chemical Name	Chemical Structure
glufosinate ammonium HOE 039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt technical is a racemic mixture of the D and L enantiomers analytical method does not distinguish between the enantiomers	
HOE 099730 IUPAC name - L-2-acetamido-4-methylphosphinico-butanoic acid analytical method can not distinguish between the D and L enantiomers	
HOE 061517 IUPAC name - 3-methylphosphinico-propionic acid	

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45204407. S. Brady (31-Mar-2000). Magnitude of Glufosinate-Ammonium Residues in or on Transgenic Rice Raw Agricultural Commodities Resulting from Two Applications of Liberty™ Herbicide, USA, 1998. Study Identification BK98R002. Unpublished

Sponsor: Aventis CropScience
Residue Chemistry Department
PO Box 538
Pikeveille, NC 27863

Executive Summary

The petitioner submitted transgenic rice magnitude of the residue data conducted in Region 10 (n=2). Liberty™ (water soluble liquid formulation; 18.2% glufosinate ammonium) was applied twice at 0.50 lbs ai/acre (total application rate of 1.00-1.02 lbs ai/acre; retreatment interval (RTI) of 14 or 24 days; 3-4 leaf stage and 3-tiller stage; spray volume - 10 gallon/acre). The field was either flooded prior to the 1st treatment or on the same day as the 1st treatment. Rice grain and rice straw were harvested at maturity 89 or 90 days after the final application and analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (all expressed as glufosinate ammonium; method was adequately validated). Combined residues of HOE 039866/HOE 099730 and HOE 061517 in/on rice grain and rice straw ranged from <0.10 - <0.16 ppm and <0.16 - 0.29 ppm, respectively (residues in/on control samples were <0.05 ppm).

A side by side comparison concerning the addition of ammonium sulfate to the spray solution was conducted at the Hamilton City, CA field trial (concentration of ammonium sulfate in the spray solution was not provided). The resulting data indicated that the addition of ammonium sulfate to the spray solution did not effect the concentrations of HOE 039866/HOE 099730 and HOE 061517 in/on rice straw and rice grain.

GLP Compliance

The in-life portion of this study was conducted by CLP Research (Chico, CA) and Agricultural Advisors (Live Oak, CA) and the analytical portion of the study was conducted by Xenos Laboratories (Ottawa, Ontario Canada). Signed and dated Good Laboratory Practices (GLP), quality assurance, and data confidentiality information were provided. The indicated deviations to the study protocol and/or GLP requirements did not effect the conclusions presented in the report.

1. Materials and Methods

1.1. Test Substance

Table 1: Active Ingredient	
Common Name:	glufosinate ammonium
IUPAC Name:	ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate
CAS Name:	butonoic acid, (±)-2-amino-4- (hydroxymethylphosphinyl)-, monoammonium salt
CAS Number:	77182-82-2
Company Name:	HOE 039866
Other Synonyms:	AE F039866, GA

1.2. Trial Locations

Table 2: Transgenic Rice Field Trial Locations[1]														
transgenic rice	Growing Region													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Submitted	-	-	-	-	-	-	-	-	-	2	-	-	-	2
Requested[2]	-	-	-	11	1	2	-	-	-	2	-	-	-	16
	-	-	-	7	1	2	-	-	-	2	-	-	-	12

[1] specific trial information, including state, crop varieties, application method and application rate and timing, can be found in Table 5

[2] second entry is for situation where a 25% reduction in the number of filed trials is possible due to residues <limit of quantitation (LOQ)

1.3. Post-harvest Procedures

The rice grain and straw samples were placed in frozen storage within 2 hours of harvest (temperature was not provided). The samples were shipped frozen via ACDS freezer truck to the AgrEvo Research Center (Pikeville, NC;). Upon arrival, the grain samples were homogenized and the resulting homogenate and the straw samples were placed in frozen storage. The grain homogenate and the straw samples were shipped via overnight delivery to Xenos Laboratories (Ottawa, Ontario) for determination of HOE 039866/HOE 099730 and HOE 061517 residues. Upon arrival at the analytical facility the samples were placed in frozen storage (-15 C). The rice grain and rice straw samples were extracted within 392 and 396 days of collection, respectively. The extracts were analyzed for residue of HOE 039866/HOE 099730 and HOE-061517 within 6 days of extraction (storage temperature was not provided).

Previously submitted and reviewed frozen storage stability data indicate that HOE 039866 and HOE 061517 are stable for 730 days on frozen apples, corn grain, and soybeans (PP#8F3607, J. Garbus, 8-Aug-1990) and 693 days on blueberries (homogenate stored for 615 days; extract stored for 78 days; 45580201.002.wpd). Additional storage stability data indicated that HOE 039866, HOE 061517, and HOE 099730 are stable for 12 months on transgenic soybean seed, forage and hay; for 3 months on soybean oil and meal; for 6 months on transgenic corn grain, fodder and forage; and for 24 months on transgenic sugar beet tops and roots (D211531 and D219069, M. Rodriguez, 7-Mar-1996; D257629, T. Bloem, 9-Jul-1999).

Based on the variety of crops tested (fruit, cereal grain, legume vegetable, root vegetable, and canola), HED concludes that these data are sufficient to validate the storage intervals and conditions for the rice straw and rice grain raw agricultural commodities (RACs) collected as part of the current study. Since the percent recoveries for fortified control samples run concurrent to the treated samples were acceptable, the storage conditions and intervals for the extracts are acceptable.

Table 3: Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (C)	Duration (days)
rice grain	homogenized RAC	stored frozen; temperature was not provided	392
	extract	temperature was not provided	1
rice straw	RAC	stored frozen; temperature was not provided	394
	extract	temperature was not provided	6

1.4. Analytical Methods

The rice grain and straw samples were analyzed for residues of HOE 039866/HOE 099730 and HOE-061517 using method BK/04/95. The method involves extraction with water, anion exchange, derivatization, silica gel column clean-up, and quantitation via gas chromatography with flame photometric detection. The dervatization step calls for the use of trimethylorthoacetate which esterifies the phosphinic and carboxylic acid function group of glufosinate, HOE 061517, and HOE 099730 and also acetylates the basic amino group of glufosinate. The analytical method does not distinguish between HOE 039866 and HOE 099730. The petitioner reported a LOQ of 0.05 ppm and a limit of detection (LOD) of 0.003 ppm. Residues in/on control samples were <0.05 ppm. The method has been adequately validated for data collection purposes.

Table 4: Percent Recovery from Fortified Control Samples.

Crop Matrix	Fortification Level (ppm)	analyte	% Recovery	Mean % Recovery ± Std Dev
rice grain	0.05	HOE 039866	91	--
		HOE 061517	85, 83	84 ± 1
		HOE 099730	90	--
	0.20	HOE 039866	96	--
		HOE 061517	87, 75	81 ± 8
		HOE 099730	78	--
rice straw	0.05	HOE 039866	69, 78	74 ± 6
		HOE 061517	98, 108, 111	106 ± 7
		HOE 099730	84	--
	0.20	HOE 039866	87	--
		HOE 061517	96, 99	98 ± 2
		HOE 099730	76	--

2. Results

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Formulation	App. Method[6]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[5]		
												HOE 039866/ HOE 099730	HOE 061517	total
Hamilton City, CA Region 10[1]	M-202[3]	Liberty™ 4	spray	0.50	3-4 leaf	1.00	14	none	grain	hand	90	<0.05, <0.05	0.11, 0.09	<0.16, <0.14
				0.50	3 tiller				straw	hand	90	<0.05, <0.05	0.13, 0.14	<0.18, <0.19
			spray	0.50	3-4 leaf	1.00	14	ammonium sulfate rate not indicated	grain	hand	90	<0.05, <0.05	0.06, 0.08	<0.11, <0.14
				0.50	3 tiller				straw	hand	90	<0.05, <0.05	0.11, 0.12	<0.16, <0.17
Live Oak, CA Region 10[2]	M-202[3]	Liberty™ 4	spray	0.51	4 leaf	1.02	24	none	grain	harvester	89	<0.05, <0.05	<0.05, 0.08	<0.10, <0.13
				0.51	3 tiller				straw	harvester	89	0.10, 0.06	0.19, 0.14	0.29, 0.20

1 field flooded 15 days prior to 1st application
2 field flooded same day as 1st application
3 M-202 = transgenic rice resistant to glufosinate ammonium; engineered to express phosphiothrion-acetyl-transferase (PAT) which enables the plant to metabolize glufosinate ammonium into a N-acetyl glufosinate (HOE 099730; not herbicidally active)
4 Liberty™ = water soluble liquid formulation; 18.2% glufosinate ammonium
5 glufosinate ammonium equivalents
6 spray volume of 10 gallons/acre

Table 6: Summary of Residue Data from Transgenic Rice Field Trials.

Commodity	Total App. Rate (lb ai/A)	PHI (days)	analyte	Residue Levels (ppm)[1]				
				Minimum	Maximum	Mean[2]	Std. Dev.[2]	HAFT[3]
Rice grain	1.00-1.02	89-90	HOE 039866/ HOE 099730	<0.05	<0.05	0.025	0	--
			HOE 061517	<0.05	0.11	0.07	0.03	--
			combined residue	<0.10	<0.16	0.10	0.03	<0.15
Rice straw	0.90	70-106	HOE 039866/ HOE 099730	<0.05	0.10	0.04	0.03	--
			HOE 061517	0.11	0.19	0.14	0.03	--
			combined residue	<0.16	0.29	0.18	0.06	0.24

1 glufosinate ammonium equivalents
2 ½ LOQ assumed for residues <LOQ
3 HAFT = highest average field trial

3. Discussion

The petitioner submitted transgenic rice magnitude of the residue data conducted in Region 10 (n=2). Liberty™ (water soluble liquid formulation; 18.2% glufosinate ammonium) was applied twice at 0.50 lbs ai/acre (total application rate of 1.00-1.02 lbs ai/acre; RTI of 14 or 24 days; 3-4 leaf stage and 3-tiller stage; spray volume - 10 gallon/acre). The field was either flooded prior to the 1st treatment or on the same day as the 1st treatment. Rice grain and rice straw were harvested at maturity 89 or 90 days after the final application and analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (all expressed as glufosinate ammonium; method was adequately validated). Combined residue of HOE 039866/HOE 099730 and HOE 061517 in/on rice grain and rice straw ranged from <0.10 - <0.16 ppm and <0.16 - 0.29 ppm, respectively (residues in/on control samples were <0.05 ppm).

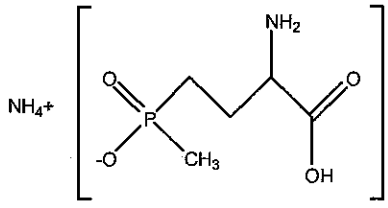
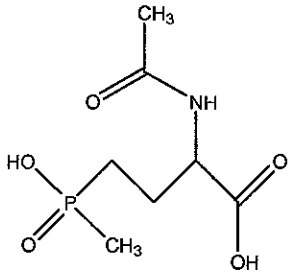
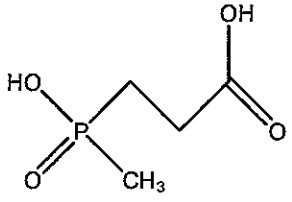
A side by side comparison concerning the addition of ammonium sulfate to the spray solution was conducted at the Hamilton City, CA field trial (concentration of ammonium sulfate in the spray solution was not provided). The resulting data indicated that the addition of ammonium sulfate to the spray solution did not effect the concentrations of HOE 039866/HOE 099730 and HOE 061517 in/on rice straw and rice grain.

4. Deficiencies

No data gaps were identified.

5. Chemical Structures

Table 7: Chemical Name and Structures

Chemical Name	Chemical Structure
glufosinate ammonium HOE 039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt technical is a racemic mixture of the D and L enantiomers analytical method does not distinguish between the enantiomers	
HOE 099730 IUPAC name - L-2-acetamido-4-methylphosphinico-butanoic acid analytical method can not distinguish between the D and L enantiomers	
HOE 061517 IUPAC name - 3-methylphosphinico-propionic acid	

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45089303. S. Dacus (30-Aug-1999). Magnitude of Glufosinate-Ammonium Residues in or on Transgenic Cotton Raw Agricultural Commodities Resulting from Two or Three Applications of Liberty™ Herbicide, USA, 1998. Study Identification BK98R005. Unpublished

Sponsor: Aventis CropScience
Residue Chemistry Department
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

Executive Summary

The petitioner submitted transgenic cotton magnitude of the residue data conducted in Region 2 (n=1), Region 3 (n=1), Region 4 (n=3), Region 6 (n=2), Region 8 (n=4), and Region 10 (n=3). Each location consisted of a control plot and two treated plots. The 1st treated plot received two over the top broadcast spray applications of glufosinate ammonium at ~0.50 lbs ai/acre (~1.00 lbs ai/acre total; retreatment interval (RTI) = 21-53 days). The 2nd treated plot received three applications of glufosinate ammonium at ~0.50 lbs ai/acre with the first and third made using over the top broadcast spray equipment and the second application directed at the bottom third of the plant (~1.50 lbs ai/acre total; RTI = 7-28 days). In all cases, glufosinate ammonium was formulated as Liberty™ (water soluble liquid formulation; 18.2% glufosinate ammonium; spray volume - 9-11 gallon/acre). Cotton was harvested by hand (n=6) or mechanically with spindle (n=4) or stripper (n=4) pickers 67-76 days after the last application. Cotton harvested by hand was ginned locally while the mechanically harvested cotton was ginned at Texas A & M University (Bryan, TX). The cottonseed and cotton gin byproduct samples were analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 (all residues expressed as glufosinate ammonium equivalents; method was adequately validated; storage interval and conditions have also been validated). Combined residues of HOE 039866/HOE 099730 and HOE 061517 in/on cottonseed treated with glufosinate ammonium at ~1.00 lbs ai/acre and ~1.50 lbs ai/acre ranged from 0.151 - 3.328 and <0.10 - 2.706 ppm, respectively (residues in/on controls

<0.05 ppm). Combined residues of HOE 039866/HOE 099730 and HOE 061517 in/on cotton gin byproducts treated with glufosinate ammonium at ~1.00 lbs ai/acre and ~1.50 lbs ai/acre ranged from 0.298 - 7.362 and 0.949 - 11.626 ppm, respectively (residues in/on controls <0.10 ppm; limit of quantiation (LOQ) = 0.10 ppm). Residue decline data has not been submitted.

GLP Compliance

The in-life portion of this study was conducted by several companies and the analytical portion of the study was conducted by AgrEvo USA (Pikeville, NC). Signed and dated Good Laboratory Practices (GLP), quality assurance, and data confidentiality information were provided. The indicated deviations to the study protocol and/or GLP requirements did not effect the conclusions presented in the report.

1. Materials and Methods

1.1. Test Substance

Table 1: Active Ingredient	
Common Name:	glufosinate ammonium
IUPAC Name:	ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate
CAS Name:	butonoic acid, (±)-2-amino-4- (hydroxymethylphosphinyl)-, monoammonium salt
CAS Number:	77182-82-2
Company Name:	HOE 039866
Other Synonyms:	AE F039866, GA

1.2. Trial Locations

Table 2: Transgenic Cotton Field Trial Locations[1]														
transgenic rice	Growing Region													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Submitted	-	1	1	3	-	2	-	4	-	3	-	-	-	14
Requested[2]	-	1	-	3	-	1	-	4	-	3	-	-	-	12
	-	1	-	2	-	1	-	3	-	2	-	-	-	9

[1] specific trial information, including state, crop varieties, application method and application rate and timing, can be found in Table 5

[2] second entry is for situation where a 25% reduction in the number of filed trials is possible due to residues <LOQ

1.3. Post-harvest Procedures

Cotton harvested by hand was ginned locally while the mechanically harvested cotton was shipped to Texas A & M University (Bryan, TX) for ginning (Rosa, LA site was mechanically harvested and ginned locally). The samples ginned locally were frozen immediately after ginning (frozen <1 - 2 days after harvest) while the samples ginned at Texas A & M University were shipped from the field at ambient temperature and were placed in frozen storage upon arrivial (ambient temperature for 1-5 days; based on information sent by P. Cain, Ph.D; Aventis Crops Science; Product Manager; 29-July-2002). The frozen ginned cottonseed and cotton gin byproduct samples were shipped via freezer truck to AgrEvo Research Center for determination of HOE 039866/HOE 099730 and HOE 061517 residues (Pikeville, NC; transport time of 39 days). The cottonseed samples were extracted within 188 days of harvest and the extracts were analyzed within 7 days of extraction. The cotton gin byproduct samples were analyzed within 218 days of harvest (interval from harvest to extraction and extraction to analysis were not provided).

Previously submitted and reviewed frozen storage stability data indicate that HOE 039866 and HOE 061517 are stable for 730 days on frozen apples, corn grain, and soybeans (PP#8F3607, J. Garbus, 8-Aug-1990) and 693 days on blueberries (homogenate stored for 615 days; extract stored for 78 days; 45580201.002.wpd). Additional storage stability data indicated that HOE 039866, HOE 061517, and HOE 099730 are stable for 12 months on transgenic soybean seed, forage and hay; for 3 months on soybean oil and meal; for 6 months on transgenic corn grain, fodder and forage; and for 24 months on transgenic sugar beet tops and roots (D211531 and D219069, M. Rodriguez, 7-Mar-1996; D257629, T. Bloem, 9-Jul-1999).

The harvested cotton samples were held at ambient temperatures for <1-5 days prior to freezing. HED concludes that this is not a unreasonable amount of time and concluded that the available storage stability data validates the storage interval and conditions for the cotton RACs. Since the percent recoveries for fortified control samples run concurrent to the treated samples were acceptable, the storage conditions and intervals for the extracts are acceptable.

Table 3: Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (C)	Duration (days)
unginned cotton	RAC	ambient	<1-5 days
cottonseed	RAC	stored frozen; temperature was not provided	188
	extract	temperature was not provided	7
cotton gin byproducts	RAC	stored frozen; temperature was not provided	218,
	extract	temperature was not provided	not provided

1.4. Analytical Methods

The cottonseed and cotton gin byproducts were analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 using method RAM BK/95/05. The cottonseed samples were extracted with 20% isopropanol while the cotton gin byproduct samples were extracted with water. The extracts were eluted through an anion exchange column, derivatized, eluted through a silica gel column, and quantified via gas chromatography with flame photometric detection (residues expressed as glufosinate ammonium equivalents). The dervatization step calls for the use of trimethylorthoacetate which esterifies the phosphinic and carboxylic acid function group of glufosinate, HOE 061517, and HOE 099730 and also acetylates the basic amino group of glufosinate. The analytical method does not distinguish between HOE 039866 and HOE 099730. The LOQ is 0.05 ppm for all analytes in/on cottonseed and cotton gin byproducts except for HOE 039866/HOE 099730 in/on cotton gin byproducts where the LOQ = 0.10 ppm. Residues in/on control samples were <LOQ. The method has been adequately validated for data collection purposes.

Table 3: Percent Recovery from Fortified Control Samples.

Matrix	Fortification Level (ppm)	% Recovery			Mean % Recovery ± Std Dev		
		HOE 039866	HOE 061517	HOE 099730	HOE 039866	HOE 061517	HOE 099730
cottonseed	0.05	74, 82	89, 105, 115, 85	117, 76	78 ± 6	98 ± 14	96 ± 29
	0.10	75	89	--	--	--	--
	1.00	78	85, 89, 96	108, 102	--	90 ± 6	105 ± 4
	2.00	--	84	106	--	--	--
	4.00	95, 81	92, 86, 83, 95	96	88 ± 10	89 ± 5	--
cotton gin byproducts	0.05	86	103	--	--	--	--
	0.10	65	82, 63, 87	115, 73	--	77 ± 13	94 ± 30
	2.00	--	62	64	--	--	--
	6.00	78	77, 71	81	--	74 ± 4	--
	15.00	91	56, 53	68	--	54 ± 2	--

2. Results

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Form.	App. Method[7]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[5]		
												HOE 039866/ HOE 099730	HOE 061517	total
Pikeville, NC Region 2	transgenic cotton[1]	Liberty™ 2	spray	0.524	4-leaf	1.047	31	none	seed	hand[3]	70	0.411, 0.731	0.054, 0.060	0.465, 0.791
			spray	0.523	early bloom				byproduct	hand[3]	70	sample not collected		
			spray	0.519	4-leaf	1.568	17, 14	none	seed	hand[3]	70	0.711, 0.842	0.106, 0.110	0.817, 0.952
			directed[6]	0.526	9-node				byproduct	hand[3]	70	sample not collected		
			spray	0.523	early bloom									
Molino, FL Region 3	transgenic cotton[1]	Liberty™ 2	spray	0.458	4-leaf	0.937	31	none	seed	hand[3]	67	0.322, 0.354	<0.05, <0.05	<0.372, <0.404
			spray	0.479	early bloom				byproduct	hand[3]	67	sample not collected		
			spray	0.458	4-leaf	1.463	19, 12	none	seed	hand[3]	67	0.349, 0.495	<0.05, <0.05	<0.399, <0.445
			directed[6]	0.526	45-55 cm				byproduct	hand[3]	67	sample not collected		
			spray	0.479	early bloom									
Rosa, LA Region 4	transgenic cotton[1]	Liberty™ 2	spray	0.519	4-6 leaf	1.034	38	none	seed	spindle[4]	70	0.523, 0.379	0.162, 0.080	0.685, 0.459
			spray	0.515	early bloom				byproduct	spindle[4]	70	sample not collected		
			spray	0.519	4-6 leaf	1.553	24, 14	none	seed	spindle[4]	70	0.446, 0.492	0.135, 0.144	0.581, 0.636
			directed[6]	0.514	8-10 leaf				byproduct	spindle[4]	70	sample not collected		
			spray	0.520	early bloom									
Greenville, MS Region 4	transgenic cotton[1]	Liberty™ 2	spray	0.522	4-leaf	1.040	35	none	seed	spindle[4]	68	1.366, 0.792	0.075, <0.05	1.441, 0.817
			spray	0.518	early bloom				byproduct	spindle[4]	68	2.377, 3.349	0.508, 0.475	2.885, 3.824
			spray	0.520	4-leaf	1.556	22, 13	none	seed	spindle[4]	68	1.603, 1.513	0.064, 0.070	1.667, 1.583
			directed[6]	0.518	41-53 cm				byproduct	spindle[4]	68	2.320, 1.853	0.624, 0.495	2.944, 2.348
			spray	0.518	early bloom									

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Form.	App. Method[7]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[5]		
												HOE 039866/ HOE 099730	HOE 061517	total
W. Memphis, AR Region 4	transgenic cotton[1]	Liberty™ 2	spray	0.514	4-leaf	1.033	53	none	seed	spindle[4]	70	3.014, 3.182	0.128, 0.146	3.142, 3.328
			spray	0.519	early bloom				byproduct	spindle[4]	70	3.013, 4.000	0.497, 0.599	3.510, 4.599
			spray	0.515	4-leaf	1.552	25, 28	none	seed	spindle[4]	70	2.533, 2.224	0.146, 0.151	2.679, 2.375
			directed[6]	0.517	squaring6				byproduct	spindle[4]	70	3.358, 4.091	0.648, 0.630	4.006, 4.721
			spray	0.520	early bloom									
Brookshire, TX Region 6	transgenic cotton[1]	Liberty™ 2	spray	0.515	4-leaf	1.037	34	none	seed	hand[3]	70	0.844, 0.516	0.110, 0.095	0.954, 0.611
			spray	0.522	early bloom				byproduct	hand[3]	70	sample not collected		
			spray	0.513	4-leaf	1.551	18, 16	none	seed	hand[3]	70	0.674, 0.500	0.068, 0.058	0.742, 0.558
			directed[6]	0.524	squaring				byproduct	hand[3]	70	sample not collected		
			spray	0.514	early bloom									
E. Bernard, TX Region 6	transgenic cotton[1]	Liberty™ 2	spray	0.515	4-leaf	1.032	40	none	seed	stripper[4]	70	0.126, 0.167	<0.05, <0.05	<0.176, <0.217
			spray	0.517	early bloom				byproduct	stripper[4]	70	1.231, 1.333	0.202, 0.215	1.433, 1.548
			spray	0.519	4-leaf	1.548	15, 25	none	seed	stripper[4]	70	0.265, 0.138	<0.05, <0.05	<0.315, <0.188
			directed[6]	0.514	fruit set				byproduct	stripper[4]	70	1.243, 1.919	0.231, 0.286	1.474, 2.205
			spray	0.515	early bloom									
Edmonson, TX Region 8	transgenic cotton[1]	Liberty™ 2	spray	0.498	4-leaf	1.024	40	none	seed	stripper[4]	69	0.353, 0.252	<0.05, <0.05	<0.403, <0.302
			spray	0.526	early bloom				byproduct	stripper[4]	69	0.273, 0.834	<0.05, 0.110	<0.323, 0.944
			spray	0.510	4-leaf	1.556	21, 19	none	seed	stripper[4]	69	0.344, <0.05	<0.05, <0.05	<0.395, <0.10
			directed[6]	0.518	squaring				byproduct	stripper[4]	69	0.827, 1.049	0.122, 0.189	0.949, 1.238
			spray	0.528	early bloom									

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Form.	App. Method[7]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[5]		
												HOE 039866/ HOE 099730	HOE 061517	total
Levelland, TX Region 8	transgenic cotton[1]	Liberty™ 2	spray	0.504	4-leaf	1.021	40	none	seed	stripper[4]	70	1.511, 1.755	0.072, 0.090	1.583, 1.845
			spray	0.517	early bloom				byproduct	stripper[4]	70	6.676, 6.896	0.452, 0.466	7.128, 7.362
			spray	0.507	4-leaf	1.538	19, 21	none	seed	stripper[4]	70	1.776, 2.383	0.177, 0.159	1.953, 2.542
			directed[6]	0.515	squaring				byproduct	stripper[4]	70	10.754, 9.563	0.872, <0.05	11.626, 9.588
			spray	0.516	early bloom									
Eakly, OK Region 8	transgenic cotton[1]	Liberty™ 2	spray	0.516	4-leaf	1.051	22	none	seed	stripper[4]	70	0.187, 0.164	0.063, 0.060	0.250, 0.224
			spray	0.535	early bloom				byproduct	stripper[4]	70	0.331, 0.345	0.269, 0.279	0.600, 0.624
			spray	0.512	4-leaf	1.560	14, 8	none	seed	stripper[4]	70	0.257, 0.199	0.699, 0.870	0.956, 1.069
			directed[6]	0.522	squaring				byproduct	stripper[4]	70	0.624, 0.602	3.214, 4.982	3.838, 5.584
			spray	0.526	early bloom									
Dill City, OK Region 8	transgenic cotton[1]	Liberty™ 2	spray	0.521	4-leaf	1.042	21	none	seed	spindle[4]	69	0.284, 0.325	<0.05, <0.05	<0.324, <0.375
			spray	0.521	early bloom				byproduct	spindle[4]	69	0.864, 0.920	0.225, 0.268	1.089, 1.188
			spray	0.522	4-leaf	1.561	14, 7	none	seed	spindle[4]	69	0.132, 0.112	0.101, 0.105	0.233, 0.217
			directed[6]	0.517	squaring				byproduct	spindle[4]	69	0.370, 0.387	0.723, 0.661	1.093, 1.048
			spray	0.522	early bloom									
Maricopa, AZ Region 10	transgenic cotton[1]	Liberty™ 2	spray	0.522	4-leaf	1.043	35	none	seed	hand[3]	76	0.880, 1.172	0.055, 0.094	0.935, 1.266
			spray	0.521	early bloom				byproduct	hand[3]	76	sample not collected		
			spray	0.530	4-leaf	1.574	19, 16	none	seed	hand[3]	76	1.290, 1.125	0.098, 0.079	1.388, 1.204
			directed[6]	0.523	squaring				byproduct	hand[3]	76	sample not collected		
			spray	0.521	early bloom									
Somerton, AZ Region 10	transgenic cotton[1]	Liberty™ 2	spray	0.521	4-leaf	1.043	36	none	seed	hand[3]	70	1.272, 1.076	0.164, 0.153	1.436, 1.229
			spray	0.522	early bloom				byproduct	hand[3]	70	sample not collected		

Table 5: Transgenic Rice Field Trial Information and Results.

Location and EPA Region	Crop and Variety	Form.	App. Method[7]	App. Rate (lbs ai/A)	App. Timing	Total app. rate (lbs ai/A)	RTI (days)	Tank Mix Adjuvants	Plant Part	Harvest Method	PHI	Residue (ppm)[5]		
												HOE 039866/ HOE 099730	HOE 061517	total
			spray	0.511	4-leaf	1.534	15, 21	none	seed	hand[3]	70	1.326, 1.524	0.155, 0.217	1.481, 1.741
			directed[6]	0.519	squaring				byproduct	hand[3]	70	sample not collected		
			spray	0.504	early bloom									
Hickman, CA Region 10	transgenic cotton[1]	Liberty™ 2	spray	0.503	4-6 leaf	1.012	41	none	seed	hand[3]	70	1.742, 2.293	0.211, 0.264	1.953, 2.557
			spray	0.509	early bloom				byproduct	hand[3]	70	sample not collected		
			spray	0.510	4-6 leaf	1.538	22, 19	none	seed	hand[3]	70	2.481, 1.313	0.225, 0.126	2.706, 1.439
			directed[6]	0.513	46 cm				byproduct	hand[3]	70	sample not collected		
			spray	0.515	early bloom									

1 LL-8B-Cotton-M, Line Cot05; engineered to express phosphiothrion-acetyl-transferase (PAT) which enables the plant to metabolize glufosinate ammonium into a N-acetyl glufosinate (HOE 099730; not herbicidally active)
2 Liberty™ = water soluble liquid formulation; 18.2% glufosinate ammonium
3 harvested by hand and ginned locally
4 harvested mechanically and ginned at Texas A & M University (Bryan, TX); except for Rosa, LA site which was ginned locally
5 glufosinate ammonium equivalents
6 directed application aimed at bottom third
7 spray volume of 9-11 gallons/acre

Table 6: Summary of Residue Data from Transgenic Rice Field Trials.								
Commodity	Total App. Rate (lb ai/A)	PHI (days)	analyte	Residue Levels (ppm)[1]				
				Min.	Max.	Mean[2]	Std. Dev.[2]	HAFT[3]
cottonseed	0.94-1.05	67-76	HOE 039866/ HOE 099730	0.126	3.182	0.928	0.838	--
			HOE 061517	<0.05	0.264	0.084	0.063	--
			combined residue	<0.176	3.328	1.012	0.881	3.235[4]
	1.46-1.57	67-76	HOE 039866/ HOE 099730	<0.05	2.533	0.967	0.790	--
			HOE 061517	<0.05	0.870	0.150	0.189	--
			combined residue	<0.10	2.706	1.117	0.803	2.527[4]
cotton gin byproducts	0.94-1.05	67-76	HOE 039866/ HOE 099730	0.273	6.896	2.317	2.232	--
			HOE 061517	<0.05	0.599	0.150	0.190	--
			combined residue	<0.323	7.362	2.645	2.357	7.244[5]
	1.46-1.57	67-76	HOE 039866/ HOE 099730	0.370	10.754	2.783	3.323	--
			HOE 061517	<0.05	4.982	0.979	1.389	--
			combined residue	0.949	11.626	3.762	3.272	10.607[5]

1 glufosinate ammonium equivalents
2 ½ LOQ assumed for residues <LOQ
3 HAFT = highest average field trial
4 W. Memphis, AR field trial
5 Levelland, TX field trail

3. Discussion

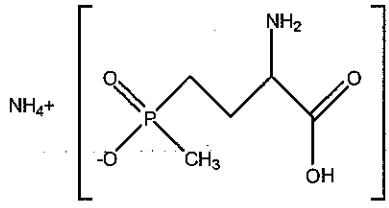
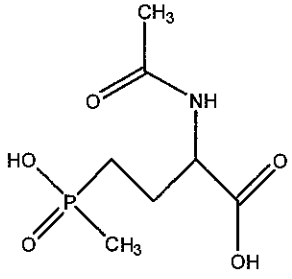
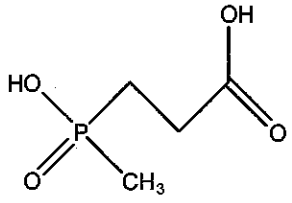
The petitioner submitted transgenic cotton magnitude of the residue data conducted in Region 2 (n=1), Region 3 (n=1), Region 4 (n=3), Region 6 (n=2), Region 8 (n=4), and Region 10 (n=3). Each location consisted of a control plot and two treated plots. The 1st treated plot received two over the top broadcast spray applications of glufosinate ammonium at ~0.50 lbs ai/acre (~1.00 lbs ai/acre total; RTI = 21-53 days). The 2nd treated plot received three applications of glufosinate ammonium at ~0.50 lbs ai/acre with the first and third made using over the top broadcast spray equipment and the second application directed at the bottom third of the plant (~1.50 lbs ai/acre total; RTI = 7-28 days). In all cases, glufosinate ammonium was formulated as Liberty™ (water soluble liquid formulation; 18.2% glufosinate ammonium; spray volume - 9-11 gallon/acre). Cotton was harvested by hand (n=6) or mechanically with spindle (n=4) or stripper (n=4) pickers 67-76 days after the last application. Cotton harvested by hand was ginned locally while the mechanically harvested cotton was ginned at Texas A & M University (Bryan, TX). The cottonseed and cotton gin byproduct samples were analyzed for residues of HOE 039866/HOE 099730 and HOE 061517 using method RAM BK/95/05 (all residues expressed as glufosinate ammonium equivalents; method was adequately validated). Combined residues of HOE 039866/HOE 099730 and HOE 061517 in/on cottonseed treated with glufosinate ammonium at ~1.00 lbs ai/acre and ~1.50 lbs ai/acre ranged from 0.151 - 3.328 and <0.10 - 2.706 ppm, respectively (residues in/on controls <0.05 ppm). Combined residues of HOE 039866/HOE 099730 and HOE 061517 in/on cotton gin byproducts treated with glufosinate ammonium at ~1.00 lbs ai/acre and ~1.50 lbs ai/acre ranged from 0.298 - 7.362 and 0.949 - 11.626 ppm, respectively (residues in/on controls <0.10 ppm; LOQ = 0.10 ppm).

4. Deficiencies

Cotton residue decline data has not been submitted.

5. Chemical Structures

Table 7: Chemical Name and Structures

Chemical Name	Chemical Structure
glufosinate ammonium HOE 039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt technical is a racemic mixture of the D and L enantiomerss; analytical method does not distinguish between the D and L enantiomers	
HOE 099730 IUPAC name - L-2-acetamido-4-methylphosphinico-butanoic acid analytical method can not distinguish between the D and L isomers	
HOE 061517 IUPAC name - 3-methylphosphinico-propionic acid	

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45204404. S. Singer (25-Aug-2000). Residues of Glufosinate-Ammonium in Crops and Soil Irrigated with Water Drained from Fields Treated with Liberty Herbicide in Louisiana and California, USA, 1997. Study Identification BK-97R-11. Unpublished

Sponsor: Aventis CropScience
Residue Chemistry Department
PO Box 538
Pikeveille, NC 27709

Executive Summary

Field trial sites were established in Rosa, LA and Porterville, CA. The trial sites were planted with transgenic rice and glufosinate ammonium was applied twice at 0.45 lbs ai/acre. In Louisiana, both applications were made to soil and the rice field was flooded 1 day after the second application. In California, both applications were made to a flooded rice field. Five, eight, and sixteen days after the second application, paddy water was used to irrigate test plots planted with grain sorghum (irrigated 71-88 days after planting), radish (irrigated 9-38 days after planting), collard (Louisiana site only; irrigated 49-60 days after planting), and lettuce (California site only; irrigated 27-38 days after planting).

Rice paddy water samples were collected on the days of irrigation and analyzed for residues of AE F039866, AE F061517, and AE F064619 (method was adequately validated). Residues in the water samples collected from the California test site (<0.003 - 0.033 ppm) were slightly higher than the residues in the water samples collected from the Louisiana test site (<0.003 - 0.019 ppm). Residues in/on control samples were ≤0.007 ppm.

Soil samples were collected form the irrigated field one day after each irrigation and analyzed for residues of AE F039866, AE F061517, and AE F064619 (surface-3"; method was adequaltey validated). Residues in the soil samples collected from the Louisiana test site (<0.003 - 0.031 ppm) when higher than the residues in the soil samples collected from the California test site (<0.003 ppm). Residues in/on control samples were ≤0.007 ppm.

Irrigated crop samples were collected 14 days after the last irrigation and at maturity and analyzed for residues of AE F039866 and AE F061517 (method was adequately validated). The petitioner indicated that the analytical method did not distinguish between AE F039866 and AE F099730 (no validation data for AE F099730 was submitted with this study). Residues were generally less <0.008 ppm at both the Louisiana and California test sites. However, residue of AE F039866 was found in/on radish top (<0.008 - 0.014 ppm), radish root (<0.008 - 0.024 ppm) and lettuce (<0.008 - 0.009 ppm) and residues of AE F061517 were found in/on grain sorghum grain (<0.008 - 0.011 ppm), grain sorghum fodder (<0.008 - 0.008 ppm), and radish top (<0.008 - 0.013 ppm). The petitioner indicated that residue in/on some control samples were >0.008 ppm (no further information was provided).

HED does not have information concerning the storage stability of the compounds analyzed in soil and water. The crop samples were analyzed within 621 days from harvest; however, the petitioner has not provided the storage temperature. These data are necessary to validate the data generated in this study.

GLP Compliance

The in-life portion of this study was conducted by Jensen Agricultural Consultants (Washington, LA) and Research for Hire (Porterville, CA) and the analytical portion of the study was conducted by Aventis CropScience (Pikeville, NC). Signed and dated Good Laboratory Practices (GLP), quality assurance, and data confidentiality information were provided. The indicated deviations to the study protocol and GLP requirements did not effect the conclusions presented in the report.

1. Materials and Methods

1.1. Test Substance

Table 1: Active Ingredient	
Common Name:	glufosinate ammonium
IUPAC Name:	ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate
CAS Name:	butonoic acid, (±)-2-amino-4- (hydroxymethylphosphinyl)-, monoammonium salt
CAS Number:	77182-82-2
Company Name:	AE F039866
Other Synonyms:	HOE 039866, GA

1.2. Trial Information

Field trial sites were established in Rosa, LA and Porterville, CA. The test sites were planted with transgenic rice and glufosinate ammonium was applied twice at 0.45 lbs ai/acre (see Table 2 for further information). In Louisiana, both applications were made to soil and the rice field was flooded 1 day after the second application. In California, the rice field was flooded prior to the first application and remained flooded thereafter. Five, eight, and sixteen days after the second application, paddy water was used to irrigate test plots planted with grain sorghum, collard, and radish (LA site) or grain sorghum, lettuce, and radish (CA site). See Table 3 for further information.

Table 2: Transgenic Crop and Field Trail Information.

Location	Crop	Formulation	App. Timing	App. Rate (lb ai/A)	Total App. Rate (lb ai/A)	RTI[1] (days)	App. Method	Tank Mix Adjuvants
Rosa, LA Region 4	transgenic rice[3]	Liberty[2]	2-4 leaf stage	0.45	0.90	15	spray	not indicated
			2-3tiller stage	0.45				
Porterville, CA Region 10	transgenic rice[3]	Liberty[2]	2-4 leaf stage	0.45	0.90	15	spray	not indicated
			2-3tiller stage	0.45				

1 RTI = retreatment interval
2 formulation and % active ingredient were not provided
3 variety was not indicated

Table 3: Irrigated Crop Summary

Location	Crop	days after planting irrigated with paddy water
Rosa, LA Region 4	grain sorghum	77, 80, 88
	collard	50, 53, 61
	radish	10, 13, 21
Porterville, CA Region 10	grain sorghum	71, 74, 82
	lettuce	27, 30, 38
	radish	27, 30, 38

1.3. Harvest and Post-harvest Procedures

Soil samples were collected from the rice field immediately after the 1st and 2nd treatments (Louisiana only). Soil samples were also collected from the irrigated fields prior to the first irrigation (Louisiana only) and 1 day after each irrigation (Louisiana and California). The soil samples were collected by taking a 12 inch core and segmenting into surface-3", 3-6" and 6-12" samples. Rice paddy water samples were collected 4, 5, 8, and 16 days after the second application. Crop samples from the irrigated field were collected prior to the first irrigation, 2 weeks after the last irrigation, and at harvest.

Soil, water, and crop samples were collected and frozen (temperature was not provided). The samples were stored frozen at the field site for 1-33 days and shipped frozen via ACDS freezer truck to Aventis CropScience (Pikeville, NC; transport took 4-27 days). The storage temperature at the analytical facility was not provided. The soil and water samples were extracted within, 357 and 943 days of collection, respectively (interval from extraction to analysis was not provided). The petitioner indicated that the sorghum grain, sorghum forage and fodder, radish top and root, lettuce, and collard were analyzed within 545, 614, 575, 549, and 596 days of collection, respectively (interval from harvest to extraction and extraction to analysis were not provided).

Previously submitted and reviewed frozen storage stability data indicate that AE F039866 and AE F061517 are stable for 730 days on frozen apples, corn grain, and soybeans (PP#8F3607, J. Garbus, 8-Aug-1990) and 693 days on blueberries (homogenate stored for 615 days; extract stored for 78 days; 45580201.002.wpd). Additional storage stability data indicated that AE F039866, AE F061517, and AE F099730 are stable for 12 months on transgenic soybean seed, forage and hay; for 3 months on soybean oil and meal; for 6 months on transgenic corn grain, fodder and forage; and for 24 months on transgenic sugar beet tops and roots (D211531 and D219069, M. Rodriguez, 7-Mar-1996; D257629, T. Bloem, 9-Jul-1999).

HED does not have information concerning the storage stability of the compounds analyzed in soil and water. The crop samples were analyzed within 614 days from harvest; however, the petitioner has not provided the storage temperature. These data are necessary to validate the data generated in this study.

Table 4: Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (C)	Duration (days)
soil	RAC	not provided	357
	extract	not provided	not provided
water	RAC	not provided	943
	extract	not provided	not provided
crop	RAC	not provided	621[1]
	extract	not provided	not provided

[1] interval from harvest to analysis

1.4. Analytical Methods

The soil samples were analyzed for residues of AE F039866, AE F061517, and AE F064619 using a modified version of BK/01/96 (residues expressed as glufosinate ammonium free acid equivalents). Briefly, residues were extracted with an aqueous $Ca(OH)_2$ solution. The resulting extract was passed through cation and chelating columns and residues were derivatized with trimethyl orthoacetate using a microwave technique. Residues were quantified via gas chromatography with flame photometric detection (limit of quantitation (LOQ) = 0.01 ppm; limit of detection (LOD) = 0.003 ppm). Residues in/on control samples were $\leq$0.007 ppm. The analytical method has been adequately validated and is appropriate for data collection purposes.

The water samples were analyzed for residues of AE F039866, AE F061517, and AE F064619. The water samples were directly derivatized with trimethyl orthoacetate using a microwave technique and residues were quantified via gas chromatography with flame photometric detection (LOQ = 0.01 ppm; LOD = 0.003 ppm; residues expressed as glufosinate ammonium free acid equivalents). Residue in/on control samples were ≤0.007 ppm. The analytical method has been adequately validated and is appropriate for data collection purposes.

The crop samples were analyzed for residues of AE F039866 and AE F061517 using method BK/05/95 (residues expressed as glufosinate ammonium free acid equivalents). Briefly, the plant material is homogenized and extracted with water. The resulting extract is passed through an anion exchange column, derivatized with trimethyl orthoacetate, and residues were quantified via gas chromatography with flame photometric detection (LOQ = 0.05 ppm; LOD = 0.008 ppm). The petitioner indicated that residues were >LOD in/on the control samples (detailed information was not provided). The analytical method can not deistinguish between HOE 039866 and HOE 099730. However no validation data was presented for HOE 099730. The analytical method has been adequately validated for determination of AE F039866 and AE F061517 and is appropriate for data collection purposes.

Table 5: Percent Recovery from Fortified Control Samples.

Matrix	Fort Level (ppm)	AE F039866		AE F061517		AE F064619	
		% Recovery	Mean ± SD	% Recovery	Mean ± SD	% Recovery	Mean ± SD
soil	0.01 (n=26)	60, 80-120, 140	95 ± 16	70-120, 130 (n=2)	104 ± 16	70-120, 130 (n=2)	106 ± 16
	0.02 (n=17)	70-100	83 ± 10	85-120, 125	104 ± 12	81-120	102 ± 11
	0.03 (n=4)	73-107	87 ± 12	83-120	100 ± 15	70-107	89 ± 16
	0.04 (n=4)	72-95	81 ± 10	95-115	106 ± 9	92-120	103 ± 12
	0.05 (n=9)	70-120, 122	97 ± 19	82-108	92 ± 10	74-104	92 ± 11
water	0.01 (n=3)	90-110	100 ± 10	90-110	97 ± 12	80-120	100 ± 20
	0.02-0.10 (n=7)	84-118	93 ± 12	84-117, 124	102 ± 14	93-105	99 ± 4
sorghum grain	0.05 (n=1)	101	--	94	--	--	--
sorghum forage	0.05 (n=1)	97	--	79	--	--	--
sorghum fodder	0.05 (n=1)	95	--	94	--	--	--
radish top	0.05 (n=1)	91	--	73	--	--	--
radish root	0.05 (n=1)	116	--	70	--	--	--
lettuce	0.05 (n=1)	69	--	82	--	--	--
collard greens	0.05 (n=1)	104	--	92	--	--	--

2. Results

Table 6: Residue Data from Soil, Water, and Irrigated Crops.

Location	Matrix	Interval	Residue (ppm)[1]		
			AE F039866	AE F061517	AE F064619
Rosa, LA Region 4	soil from rice field; surface-3"[2]	after 1st app. (0-day)	0.229-0.279	<LOD-0.014	nd
		after 2nd app. (0-day)	0.330-0.435	0.075-0.115	0.012-0.035
	soil form irrigated field; surface-3"[2]	1-day after 1st irrigation	nd-0.031	nd-<LOD	nd-0.003
		1-day after 2nd irrigation	nd-0.009	nd-0.004	nd-<LOD
		1-day after 3rd irrigation	nd-0.003	nd-<LOD	nd
	rice paddy water	4-days after 2nd app.	0.016, 0.019	0.008, 0.010	nd
		5-days after 2nd app.	0.003, 0.006	0.005, 0.005	nd
		8-days after 2nd app.	nd	<LOD, <LOD	nd
		16-days after 2nd app.	nd	<LOD, <LOD	nd
	grain sorghum, grain	42 days after last irrigation	nd	nd, 0.011	na
	grain sorghum, forage	14 days after last irrigation	nd	nd	na
	grain sorghum, fodder	42 days after last irrigation	nd	nd, 0.008	na
	radish top	14 days after last irrigation	nd, 0.014	nd	na
	radish root	14 days after last irrigation	nd	nd, 0.016	na
	collards	14 days after last irrigation	nd	nd	na
Porterville, CA Region 10	soil form irrigated field; surface-3"[2]	1-day after 1st irrigation	nd	nd-<LOD	nd
		1-day after 2nd irrigation	nd	nd	nd
		1-day after 3rd irrigation	nd	nd	nd
	rice paddy water	after 1st app. (0-day)	0.108, 0.171	0.010, 0.010	nd
		after 2nd app. (0-day)	0.060, 0.246	0.005, 0.011	nd
		5-days after 2nd app.	0.021-0.033	0.015-0.019	nd
		8-days after 2nd app.	0.011, 0.019	0.015, 0.019	0.004, 0.004
		16-days after 2nd app.	nd, <LOD	<LOD, <LOD	nd, 0.004
	grain sorghum, grain	49 days after last irrigation	nd	nd	na
	grain sorghum, forage	14 days after last irrigation	nd	nd	na
	grain sorghum, fodder	49 days after last irrigation	nd	nd	na
	radish top	14 days after last irrigation	nd, 0.009	nd, 0.013	na
		46 days after last irrigation	nd	nd	na
	radish root	14 days after last irrigation	nd	nd	na
		46 days after last irrigation	nd, 0.024	nd	na
	lettuce	14 days after last irrigation	nd	nd	na
		46 days after last irrigation	<LOD, 0.009	nd	na

[1] glufosinate free acid equivalents
[2] residue of AE F039866, AE F061517, and AE F064619 were <LOQ in 3-6" and 6-12" segments

3. Discussion

Field trial sites were established in Rosa, LA and Porterville, CA. The test sites were planted with transgenic rice and glufosinate ammonium was applied twice at 0.45 lbs ai/acre. In Louisiana, both applications were made to soil and the rice field was flooded 1 day after the second application. In California, the rice field was flooded prior to the first application and remained flooded thereafter. Five, eight, and sixteen days after the second application, paddy water was used to irrigate test plots planted with grain sorghum (irrigated 71-88 days after planting), radish (irrigated 9-38 days after planting), collard (Louisiana site only; irrigated 49-60 days after planting), and lettuce (California site only; irrigated 27-38 days after planting).

Rice paddy water samples were collected on the days of irrigation and analyzed for residues of AE F039866, AE F061517, and AE F064619 (method was adequately validated). Residues in the water samples collected from the California test site (<0.003 - 0.033 ppm) were slightly higher than the residues in the water samples collected from the Louisiana test site (<0.003 - 0.019 ppm). Residues in/on control samples were ≤0.007 ppm. Residues in/on control samples were ≤0.007 ppm.

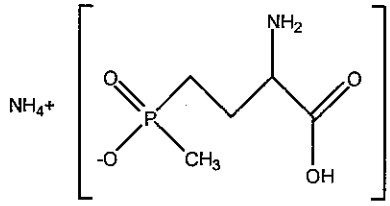
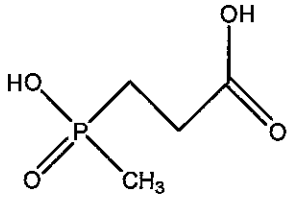
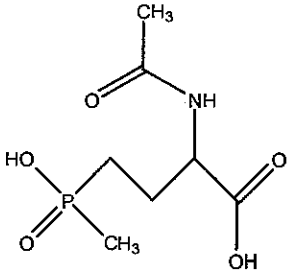
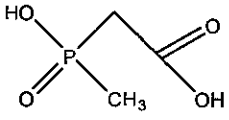
Soil samples were collected form the irrigated field one day after each irrigation and analyzed for residues of AE F039866, AE F061517, and AE F064619 (surface-3"; method was adequately validated). Residues in the soil samples collected from the Louisiana test site (<0.003 - 0.031 ppm) when higher than the residues in the soil samples collected from the California test site (<0.003 ppm). Residues in/on control samples were ≤0.007 ppm.

Irrigated crop samples were collected 14 days after the last irrigation and at maturity and analyzed for residues of AE F039866 and AE F061517 (method was adequately validated). The petitioner indicated that the analytical method did not distinguish between AE F039866 and AE F099730 (no validation data for AE F099730 was submitted with this study). Residues were generally less <0.008 ppm at both the Louisiana and California test sites. However, residues of AE F039866 were found in/on radish top (<0.008 - 0.014 ppm), radish root (<0.008 - 0.024 ppm) and lettuce (<0.008 - 0.009 ppm) and residues of AE F061517 were found in/on grain sorghum grain (<0.008 - 0.011 ppm), grain sorghum fodder (<0.008 - 0.008 ppm), and radish top (<0.008 - 0.013 ppm). The petitioner indicated that residue in/on some control samples were >0.008 ppm (no further information was provided).

4. Deficiencies

HED does not have information concerning the storage stability of the compounds analyzed in soil and water. The crop samples were analyzed within 614 days from harvest; however, the petitioner has not provided the storage temperature. These data are necessary to validate the data generated in this study.

5. Chemical Structures

Chemical Name	Chemical Structure
glufosinate ammonium AE F039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt technical is a racemic mixture of the D and L enantiomers	
AE F061517 IUPAC name - 3-methylphosphinico-propionic acid	
AE F099730 IUPAC name - L-2-acetamido-4-methylphosphinico-butanoic acid analytical method did not distinguish between D and L enantiomers; therefore, both enantiomers will be assumed to be present	
AE F064619 2-methylphosphinico-acetic acid	

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45204405; J. K. Rupprecht; 22-Aug-2000; Metabolism of [^{14}C]-Glufosinate-ammonium in Rice; Study Identification: 519BK; Unpublished Study

Sponsor: Aventis CropScience
Environmental Chemistry Department
PO Box 538
Pikeville, NC 27863

Executive Summary

The transgenic rice used in this study was engineered to express phosphinothricin-N-acetyl-transferse (PAT) enzyme; PAT acetylates glufosinate to form N-acetyl glufosinate which is not herbicidally active. The standard used to identify N-acetyl glufosinate contained only the L-enantiomer (AE F099730). Since the analytical method did not distinguish between the L and D enantiomers of N-acetyl glufosinate, it will be assumed that both are present.

Transgenic rice was treated with two applications of [3,4-^{14}C]-glufosinate ammonium at 0.46 lbs ai/acre (2-4 leaf stage and 2-4 tiller stage; 19 day retreatment interval (RTI)). Whole plant samples were collected immediately after the first application (70.10-109.44 ppm) and one day prior to the second application (1.43-3.84 ppm). Rice straw (6.72-19.43 ppm), rice stubble (material above the soil but below flood level; 4.01-13.70 ppm), and rice grain (1.12-1.36) were harvested at maturity 184 days after the second application. The majority of the total radioactive residues (TRRs) were extractable with water or acetonitrile (ACN):water (81-99% TRR). The major residues identified in the whole plant, rice straw, rice stubble, and rice grain samples were AE F039866 (6-62% TRR), AE F061517 (1-70% TRR), and AE F099730 (11-60% TRR). AE F039866 was the major residue found in/on the 0-day whole plant samples (62% TRR) while AE F099729 was the major residue in/on 18-day whole plant, rice straw, and rice stubble samples (55-60% TRR). The major residue in/on rice grain was AE F061517 (70% TRR). The nature of the residue for the transgenic rice used in this study is adequately understood.

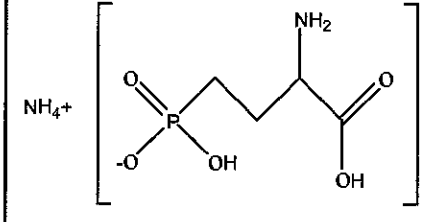
The petitioner also collected rice paddy water (7, 18, and 102 days after first application) and soil (7, 18, 102, and 202 days after the first application) samples from the rice field. TRRs in water dropped from 0.53-0.67 ppm 7 days after the first application to 0.03 ppm 18 days after the first application and were ≤0.01 ppm 83 days after the second application (102 days after the first application). The major residue identified in the day-7 water samples were AE F039866 (37% TRR) and AE F061517 (48% TRR) while the major residues identified in the day-18 samples were AE F061517 (21% TRR), AE F084658 (34% TRR), and AE F0015081 (23% TRR). TRRs in soil were relatively consistent throughout the study (0.01-0.20 ppm) with a slight increase noted for the day-202 sample most likely due to the desication of soil prior to harvest. The major residues identified in all of the soil samples were AE F039866 (4-30% TRR) and AE F061517 (18-48% TRR).

GLP Compliance

The in-life and analytical portions of this study were conducted by Aventis Crop Science Environmental Chemistry Department (Pikeville, NC). Signed and dated GLP, quality assurance, and data confidentiality information were provided. The indicated deviations from the study protocol did not affect the quality or integrity of the data.

1. Materials and Methods

1.1. Substance

Table 1: Test Substance	
Common Name	glufosinate ammonium
IUPAC Name	ammonium-DL-homoalanin-4-yl(methyl)phosphinate
CAS Name	(±)-2-amino-4-(hydroxymethylphosphinyl)butanoic acid monoammonium salt
CAS Number	77182-82-2
Company Name	AE F039866
Other Synonyms	HOE 039866, GA
Purity of Non-Labeled Material	99.2%
Radiochemical Purity of Labeled Material	>98%
Location of Isotopic Label	carbons 3 and 4
Specific Activity	specific activity of the standard - 51.8 µCi/mg; specific activity of the applied material - 45.0 µCi/mg
Structure	

1.2. Crop and Site

Table 2: Crop and Site	
Type and Variety of Crop	transgenic rice (var. Taipei); the engineered plant express the phosphinothricin-N-acetyl-transferse (PAT) enzyme; PAT confers resistance by acetylating glufosinate and thereby deactivating the herbicidal activity
Growth Environment	greenhouse; stainless steel tank (76 cm x 91 cm x 60 cm deep)
Conditions	the stainless steel tanks were filled to a depth of 4 inches with crushed rock and then with a 12 inch layer of sandy loam; temperature ranged from 15 - 40 C; plants were irrigated as needed by watering at the soil level; two water management practices were employed Tank A: rice was flooded 48 hours prior to the first application Tank B: rice was flooded 24 hours after the second application in both cases the flood water was maintained until 31 days prior to harvest

1.3. Application

Table 3: Application	
Type of Application	hand sprayer
Application Matrix	water; blank formulation
Application Rate	0.46 lbs acid equivalents per acre (ae/acre)
Number of Applications	2
Timing of Applications	2-4 leaf stage and 2-4 tiller stage (19 day RTI)

1.4. Harvest/Post-harvest Procedures

Whole Plant: Three whole plant samples were collected from each tank immediately after the first application (2-4 leaf stage) and one day prior to the second application (2 - 4 tiller stage; 18 days after the first application).

Rice Grain, Rice Straw, and Rice Stubble: Grain and straw samples were collected at maturity 184 days after the second application. The panicle was removed from the grain and combined with the straw. For this study, straw was defined as the material above the water line. The material below the water line but above the soil surface was collected and called stubble.

Water and Soil: Soil and water samples were collected from the rice field 7, 18, 102, and 202 days after the first application.

Storage of Plant, Water, and Soil Samples: The plant samples were immediately ground and stored at -15 C. The water and soil samples were immediately stored at -15 C upon collection. Preliminary chromatographic analysis was completed within 2 months of harvest. Final analysis was complete within 6 months of harvest. The petitioner indicated that the HPLC and TLC chromatographic profiles for a samples analyzed within 10 days of harvest and within 6 months of harvest were essentially identical (the chromatograms were not provided). Since the samples were stored frozen and analyzed within 6 months of harvest, storage stability data is not necessary (OPPTS 860.1380).

Table 4. Summary of Storage Conditions

Matrix	RAC or Extract	Storage Temperature (C)	Duration (days or months)
whole plant	RAC	-15	maximum of 6 months from harvest
rice straw			
rice grain			
rice stubble			
water			
soil			

1.4. Analytical Methods

Since there were no quantitative or qualitative differences in the TRR or in the metabolic profiles of rice samples collected from the two water management practices tested, Tables 5 and 6 presents data form each together.

TRR: The plant samples were ground and subjected to combustion LSC analysis for determination of TRRs. TRRs in the water samples and in extracts were determined via LSC analysis. TRRs in the day-0 plant, day-18 plant, and soil samples were determined by summing the extractable and nonextractable residues. The limit of quantitation (LOQ) was 0.003 ppm. Table 5 is a summary of the TRR found in the collected samples.

Identification/Characterization of Day-0 Rice Forage TRRs - The day-0 samples were rinsed with water (22-29% TRR). The rinsed samples were homogenized and extracted with water (68-76% TRR). The water rinse and the water extract were HPLC/TLC analyzed. The post-extraction solids (PESs) were not further analyzed (1-3% TRR).

Identification/Characterization of Day-18 Rice Forage TRRs - The day-18 samples were rinsed with water (<1-6% TRR). The rinsed samples were homogenized and extracted with water (70-86% TRR) and ACN (<1-6% TRR). The water extract was HPLC/TLC analyzed. The PESs were not further analyzed (10-20% TRR).

Identification/Characterization of Rice Straw TRRs - The rice straw samples were homogenized and extracted with water (72-87% TRR) and ACN:water (1:1; 7-22% TRR). The water extract was filtered (72-87% TRR) and HPLC/TLC analyzed. The ACN:water extracts were reduced to the aqueous phase, freeze dried, reconstituted in water, and centrifuged. The supernatant was collected (7-22% TRR) and HPLC/TLC analyzed. The PESs were not further analyzed (5-6% TRR)

Identification/Characterization of Rice Stubble TRRs - The rice stubble samples were homogenized and extracted with water (79-84% TRR) and ACN:water (1:1; 7-14% TRR). The water extract was filtered (79-84% TRR) and HPLC/TLC analyzed. The ACN:water extracts were reduced to the aqueous phase, freeze dried, reconstituted in water, and centrifuged. The supernatant was collected, filtered (9-14% TRR), and HPLC/TLC analyzed. The PESs were not further analyzed (5-10% TRR).

Identification/Characterization of Rice Grain TRRs - The rice grain samples were homogenized and extracted with water (83-89% TRR) and ACN:water (1:1; 4-6% TRR). The water and the ACN:water extracts were combined, freeze dried, reconstituted in water, and centrifuged. The supernatant was collected (82-91% TRR) and HPLC/TLC analyzed. The extracted rice grain was mixed with 1M ethanolic potassium hydroxide for 24 hours at 50 C. The resulting mixture was centrifuged and the supernatant collected and combined with a post-hydrolysis aqueous wash (5-6% TRR). The combined supernatant and aqueous wash were neutralized with 5 M HCl, reduced via rotary evaporation, and HPLC/TLC analyzed. The PESs were not further analyzed (8-9% TRR).

Identification/Characterization of Water TRRs (day-7, day-18, and day-102) - The day-7 and day-18 water samples were centrifuged and the supernatant was directly HPLC/TLC analyzed. Day-102 samples were not analyzed (TRR ≤0.01 ppm).

Identification/Characterization of Soil TRRs (day-7, day-18, and day-102) - The soil samples were centrifuged and the aqueous supernatant was collected (22-57% TRR). The soil was washed with water, centrifuged, and the water collected (13-47% TRR). The aqueous supernatant and extract were HPLC/TLC analyzed. The PESs were not further analyzed (12-72% TRR).

Identification/Characterization of Soil TRRs (day-202) - The soil samples were centrifuged and the supernatant was collected (8-41% TRR; not further analyzed). The soil was washed with water, centrifuged, and the water collected (15-23% TRR). The water extract was freeze dried, reconstituted in water (15-23% TRR), and HPLC/TLC analyzed. The extracted soil was mixed with 10% HCl/dioxane at room temperature for 16 hours. The resulting mixture was centrifuged and the supernatant collected (27-50% TRR). The PESs were not further analyzed (15-48% TRR).

HPLC/TLC Analysis - The HPLC was fitted with a Phenomenex Sphereclone SAX column (4.6 x 250 mm). The column was maintained at ambient temperature throughout the run. The mobile phase consisted of a 0.02 M potassium dihydrogen phosphate buffer solution (flow rate 1.0 ml/minute). Fractions eluting from the HPLC column were collected in one minute intervals and quantified via LSC analysis. Residues were identified based on retention time of the following standards (see Section 5 for structures): AE F039866, AE F061517, AE F099730, AE F064619, AE F084658, and AE 0015081. Residue identification was confirmed via TLC analysis. The TLC system consisted of Machery-Nagel Sil G25 plated and a isoprorpanol:water:acetic acid (2:1:1) normal phase solvent system. Although standard AE F099730 is only the L-enantiomer, the analytical method can not distinguish between the D and L enantiomers.

2. Results

Table 5: TRR in Rice, Water, and Soil

matrix	TRR (ppm)[1]
day-0 rice forage	70.10 - 109.44
day-18 rice forage	1.43 - 3.84
rice straw	6.72 - 19.43
rice stubble	4.01 - 13.70
rice grain	1.12 - 1.36
day-7 water	0.56-0.67
day-18 water	0.03
day-102 water	≤0.01
day-7 soil	0.04 - 0.22
day-18 soil	0.01 - 0.09
day-102 soil	0.03 - 0.09
day-202 soil	0.12-0.20

[1] glufosinate ammonium equivalents

Table 6: Identification/Characterization of Radioactive Residues in Rice, Water, and Soil

matrix TRR (ppm)	residue distribution	%TRR, ppm	residue identification (% TRR, ppm[2])						
			AE F039866	AE F061517	AE F099730	AE F064619	AE F084658	AE 0015081	unknowns
day-0 rice forage 70.10-109.44	surface wash	22-29%, 20.28-29.83	20-27%, 17.21-28.38	nd	nd	nd	nd	nd	≤4%, ≤2.58
	water extract	68-76%, 48.70-77.20	33-43%, 23.40-46.64	≤1%, 0.63-1.28	23-34%, 22.31-30.56	nd	≤1%, ≤0.78	nd	≤1%, ≤0.34
	PES	1-3%, 0.80-2.41	not analyzed						
	total identified[1]	93%, 81.12	62%, 54.66	1%, 0.85	30%, 25.40	nd	≤1%, 0.54	nd	≤4, ≤2.58
day-18 rice forage 1.43-3.84	surface wash	<1-6%, <0.01-0.12	not analyzed						
	water extract	70-86%, 1.21-3.31	8%, 0.12-0.31	6-10%, 0.12-0.37	54-66%, 0.88-2.52	<1%, nd-0.006	≤1%, <0.01-0.02	nd	≤2%, ≤0.02
	ACN extract	<1-6%, <0.01-0.14	not analyzed						
	PES	10-20%, 0.20-0.45	not analyzed						
	total identified[1]	80%, 1.82	8%, 0.18	8%, 0.20	59%, 1.40	<1%, <0.01	<1%, 0.01	nd	≤2%, ≤0.02
rice straw 6.72-19.43	water extract	72-87%, 5.86-16.99	14-17%, 1.04-3.28	8-14%, 0.74-2.15	47-56%, 3.65-10.91	2-3%, 0.17-0.31	nd	nd	≤4%, ≤0.31
	ACN:water ext	7-22%, 0.45-2.37	1-4%, 0.07-0.40	1-2%, 0.07-0.26	4-14, 0.26-1.50	≤1, 0.01-0.08	nd	nd	<1%,≤0.08
	PES	5-6%, 0.41-0.92	not analyzed						
	total identified[1]	91%, 10.33	18%, 2.00	12%, 1.30	60%, 6.78	2%, 0.26	nd	nd	≤4%, ≤0.31
rice stubble 4.01-13.70	water extract	79-87%, 3.25-16.99	8-10%, 0.30-1.30	13-31%, 1.24-1.90	36-54%, 1.43-7.10	1-2%, 0.07-0.16	nd	nd	≤4%, ≤0.33
	ACN:water ext	7-14%, 0.36-1.90	1-2%, 0.03-0.24	2-3%, 0.14-0.33	4-9%, 0.15-1.20	<1%, 0.01-0.02	nd	nd	≤1%, ≤0.09
	PES	5-10%, 0.40-1.00	not analyzed						
	total identified[1]	88%, 8.09	10%, 0.97	21%, 1.69	55%, 5.36	2%, 0.14	nd	nd	≤4%, ≤0.33
rice grain 1.12-1.36	water extract	83-89%, 0.93-1.21	4-5%, 0.04-0.07	64-74%, 0.72-1.00	10-12%, 0.14-0.17	≤1%, <0.01-0.01	nd	nd	≤2%, ≤0.02
	ACN:water ext	4-6%, 0.04-0.09							
	hydrolysate	5-6%, 0.06	2%, 0.02	3%, 0.03-0.04	<1%, <0.01	<1%, <0.01	nd	nd	<1%, <0.01
	PES	8-9%, 0.09-0.10	not analyzed						
	total identified[1]	88%, 1.09	6%, 0.07	70%, 0.88	11%, 0.14	1%, 0.01	nd	nd	≤2%, ≤0.02

Table 6: Identification/Characterization of Radioactive Residues in Rice, Water, and Soil

matrix TRR (ppm)	residue distribution	%TRR, ppm	residue identification (% TRR, ppm[2])						
			AE F039866	AE F061517	AE F099730	AE F064619	AE F084658	AE 0015081	unknowns
day-7 water 0.56-0.67	analyzed directly	100%, 0.56-0.67	36-37%, 0.20-0.25	45-49%, 0.27-0.33	4-5%, 0.03	nd	2-3%, 0.01-0.02	7%, 0.04-0.05	≤2%, ≤0.01
	total identified[1]	98%, 0.62	37%, 0.23	48%, 0.30	4%, 0.03	nd	2%, 0.01	7%, 0.04	≤2%, ≤0.01
day-18 water 0.03-0.04	analyzed directly	100%, 0.03-0.04	5%, <0.01	14-29%, <0.01-0.01	4%, <0.01	<1-5%, <0.01	33-34%, 0.01	21-25%, <0.01	≤6%, ≤0.01
	total identified[1]	90%, 0.03	5%, <0.01	21%, <0.01	4%, <0.01	3%, <0.01	34%, 0.01	23%, <0.01	≤6%, ≤0.01
day-102 water; <0.01-0.01 ppm; not analyzed									
day-7 soil 0.04-0.22	water centrifuge	40-57%, 0.03-0.09	2-20%, <0.01-0.02	22-34%, 0.01-0.08	2-4%, <0.01	nd	<1-2%, <0.01	3-5%, <0.01	≤4%, <0.01
	water ext	30-40%, 0.01-0.08	3-14%, <0.01-0.01	15-29%, <0.01-0.06	1-4%, <0.01	<1-2%, <0.01	<1-2%, <0.01	<1-2%, <0.01	≤1%, <0.01
	PES	12-21%, <0.01-0.05	not analyzed						
	total identified[1]	80%, 0.09	18%, 0.02	48%, 0.06	5%, <0.01	2%, <0.01	2%, <0.01	5%, <0.01	≤4%, <0.01
day-18 soil <0.01-0.09	water centrifuge	22-42%, 0.02-0.03	1-2%, <0.01	16-33%, 0.02	1-2%, <0.01	nd	2%, <0.01	3-4%, <0.01	<1%, <0.01
	water ext	28-47%, <0.01-0.03	2-3%, <0.01	22-24%, 0.02	2%, <0.01	nd	<1%, <0.01	2-3%, <0.01	<1%, <0.01
	PES	24-72%, <0.01-0.04	not analyzed						
	total identified[1]	63%, 0.05	4%, <0.01	48%, 0.04	4%, <0.01	nd	2%, <0.01	6%, <0.01	<1%, <0.01
day-102 soil 0.03-0.09	water centrifuge	25-53%, <0.01-0.03	2-3%, <0.01	16-36%, <0.01-0.02	1-4%, <0.01	nd	<1-9%, <0.01	<1-1%, <0.01	<1%, <0.01
	water ext	13-18%, <0.01-0.02	2%, <0.01	8%, <0.01	5%, <0.01	nd	3%, <0.01	<1%, <0.01	<1%, <0.01
	PES	31-61%, 0.01-0.05	not analyzed						
	total identified[1]	42%, 0.02	4%, <0.01	26%, 0.01	5%, <0.01	nd	6%, <0.01	<1%, <0.01	<1%, <0.01
day-202 soil 0.12-0.20	water centrifuge	8-41%, 0.01-0.08	not analyzed						
	water ext	15-23%, 0.02-0.03	1-4%, <0.01	9-16%, 0.01-0.02	<1-2%, <0.01	<1%, <0.01	<1-1%, <0.01	<1%, <0.01	<1%, <0.01
	hydrolysate	27-50%, 0.04-0.07	14-42%, 0.02-0.06	1-11%, <0.01-0.02	<1-2%, <0.01	<1-2%, <0.01	<1-1%, <0.01	<1-2%, <0.01	≤1%, <0.01
	PES	15-48%, 0.02-0.06	not analyzed						
	total identified[1]	54%, 0.08	30%, 0.04	18%, 0.03	2%, <0.01	1%, <0.01	1%, <0.01	<1%, <0.01	≤1%, <0.01

nd not detected
[1] mean values were summed
[2] glufosinate ammonium equivalents

3. Discussion

The transgenic rice used in this study was engineered to express phosphinothricin-N-acetyl-transferse (PAT) enzyme; PAT acetylates glufosinate to form N-acetyl glufosinate which is not herbicidally active. The standard used to identify N-acetyl glufosinate contained only the L-enantiomer (AE F099730). Since the analytical method did not distinguish between the L and D enantiomers of N-acetyl glufosinate, it will be assumed that both are present.

Transgenic rice was treated with two applications of [3,4-^{14}C]-glufosinate ammonium at 0.46 lbs ai/acre (2-4 leaf stage and 2-4 tiller stage; 19 day RTI). Whole plant samples were collected immediately after the first application (70.10-109.44 ppm) and one day prior to the second application (1.43-3.84 ppm). Rice straw (6.72-19.43 ppm), rice stubble (material above the soil but below flood level; 4.01-13.70 ppm), and rice grain (1.12-1.36 ppm) were harvested at maturity 184 days after the second application. The harvested whole plant, straw, stubble, and grain samples were extracted with water or ACN:water (81-99% TRR). The PESs of whole plant (1-20% TRR), straw (5-6% TRR), and rice stubble (5-10% TRR) were not further characterized. The rice grain PESs were hydrolyzed with potassium hydroxide and the hydrolysate was collected and combined with a post-hydrolysis aqueous wash (5-6% TRR, PESs were not further analyzed (8-9% TRR)). The extracts and hydrolysate were HPLC/TLC analyzed with 80-93% of the TRR identified. The major residues identified were AE F039866 (6-62% TRR), AE F061517 (1-70% TRR), and AE F099730 (11-60% TRR). Minor amounts of AE F064619 and AE F084658 (≤2% TRR) were also identified (unknowns ≤4% TRR). AE F039866 was the major residue found in/on the 0-day whole plant samples (62% TRR) while AE F099729 was the major residue in/on 18-day whole plant, rice straw and rice stubble samples (55-60% TRR). The major residue in/on rice grain was AE F061517 (70% TRR). The nature of the residue for the transgenic rice used in this study is adequately understood.

The petitioner collected water and soil samples from the rice field 7 (water - 0.56-0.67 ppm; soil - 0.04-0.22 ppm), 18 (water - 0.03 ppm; soil - 0.01-0.09 ppm), 102 (83 days after second application; water - ≤0.01 ppm; soil - 0.03-0.09 ppm) and 202 (183 days after the second application; water - not collected; soil - 0.12-0.20 ppm) days after the first application.

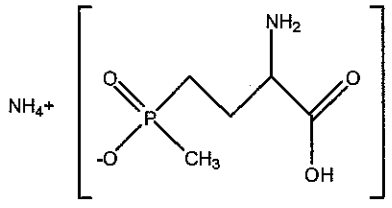
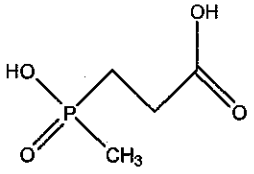
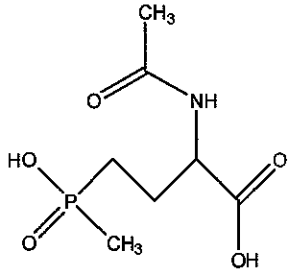
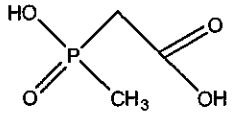
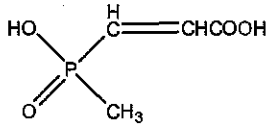
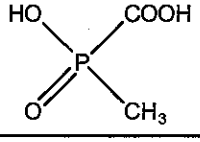
The water samples were centrifuged and the resulting supernatant was HPLC/TLC analyzed (90-92% TRR identified, day-102 water samples were not analyzed TRR ≤ 0.01 ppm). The major residue identified in the day-7 samples were AE F039866 (37% TRR) and AE F061517 (48% TRR) with minor quantities of AE F099729, AE F084658, and AE F0015081 also found (non-detect-7% TRR). The major residues identified in the day-18 samples were AE F061517 (21% TRR), AE F084658 (34% TRR), and AE F0015081 (23% TRR) with minor quantities of AE F039866, AE F099729, and AE F064619 also found (≤5% TRR; unknowns ≤6% TRR).

The soil samples were centrifuged (supernatant - 22-53% TRR) and extracted with water (13-47% TRR). The PESs for the day-7, day-18, and day-102 soil samples were not further analyzed (12-61% TRR, ≤0.05 ppm). The PESs for the day-202 soil samples were hydrolyzed with HCl/dioxane and the resulting hydrolysate collected (27-50% TRR; PESs were not further analyzed (15-48% TRR; ≤0.06 ppm)). The extracts and hydrolysate were HPLC/TLC analyzed with 42-80% of the TRR identified. The major residues identified in all of the soil samples were AE F039866 (4-30% TRR) and AE F061517 (18-48% TRR) with minor quantities of AE F099729, AE. F064619, AE F084658, and AE F 0015081 also found (≤6% TRR; unknowns ≤4% TRR).

4. Deficiencies

No data gaps were identified in this study.

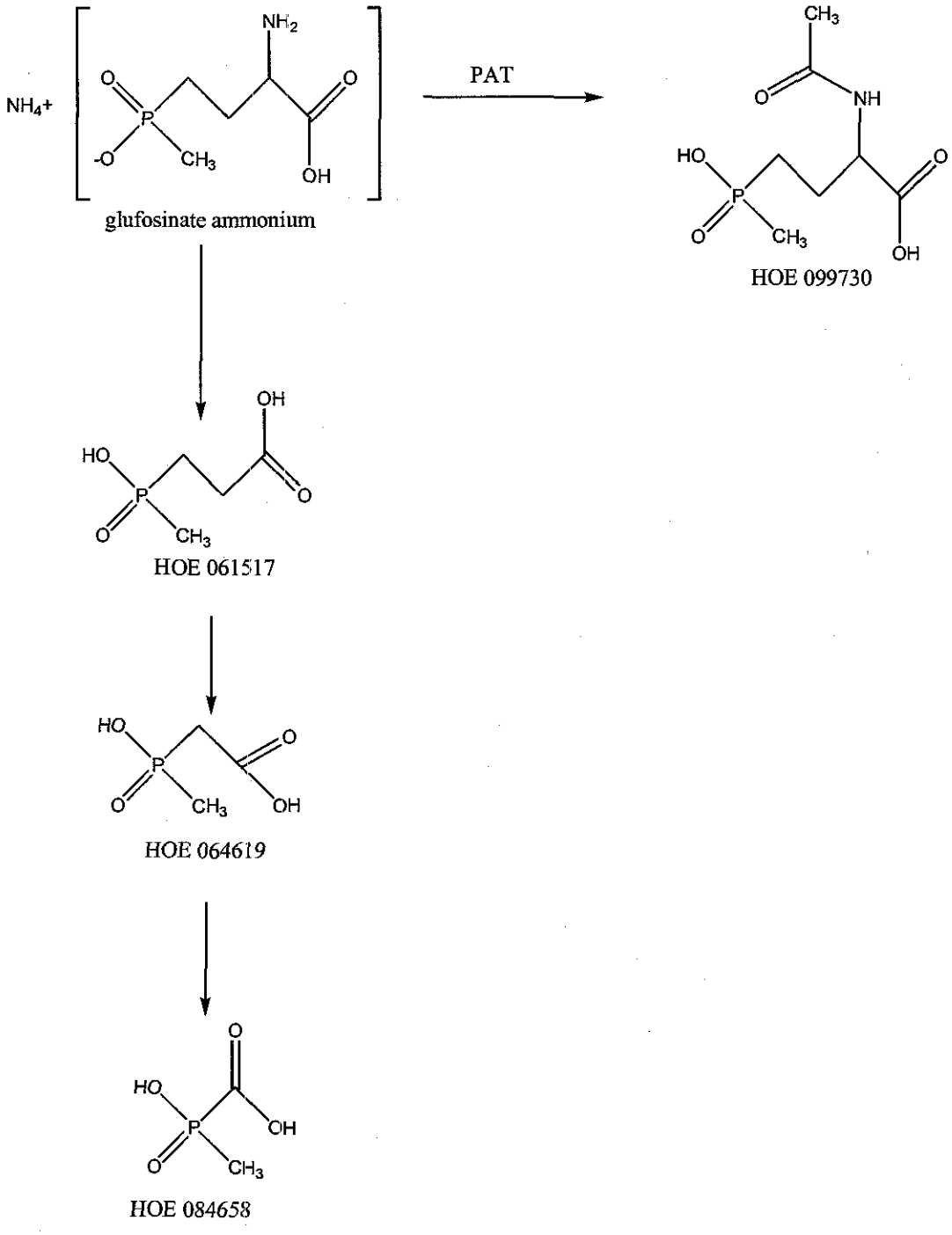
5. Structures

Chemical Name	Chemical Structure
glufosinate ammonium AE F039866 CAS name - butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt technical is a racemic mixture of the D and L enantiomers; analytical method does not distinguish between the two enantiomers	
AE F061517 IUPAC name - 3-methylphosphinico-propionic acid	
AE F099730 IUPAC name - L-2-acetamido-4-methylphosphinico- butanoic acid analytical method did not distinguish between D and L enantiomers; therefore, both enantiomers will be assumed to be present	
AE F064619 2-methylphosphinico-acetic acid	
AE 0015081	
AE F084658	

attachment 1: petitioner proposed metabolic pathway

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

Attachment 1: Petitioner's Proposed Metabolic Pathway



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 20-June-2002

Reviewers: Tom Bloem, Chemist
Registration Action Branch 1, Health Effects Division (RAB1/HED)

G. Jeffrey Herndon, Branch Senior Scientist
RAB1/HED

DP Barcode: Residue Chemistry Summary Memorandum - D271110, T. Bloem, 20-June-2002

Citation: MRID 45580201. F. Salzman (7-Jan-2002). Glufosinate-Ammonium: Magnitude of the Residue on Blueberry. Study Number 05291. Unpublished

Sponsor: IR-4 Project
Rutgers, The State University of New Jersey
681 U.S. Highway 1 South
North Brunswick, NJ 08902-3390

Executive Summary

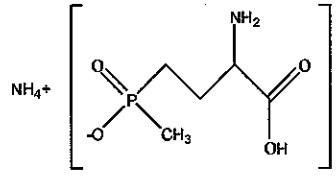
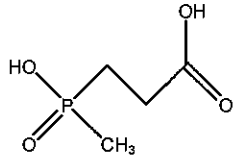
Control blueberry samples were fortified with HOE 039866 and HOE 061517 at 1.00 ppm and placed in frozen storage (<-20 C). The samples were extracted after 615 (HOE 039866) or 593 (HOE 061517) days of storage and the resulting extracts were analyzed 78 (HOE 039866) or 71 (HOE 061517) days after extraction (extract was stored at <-20 C). The resulting percent recoveries for HOE 039866 (95, 96, 98) and HOE 061517 (73, 72, 72) were acceptable.

GLP Compliance

The study was conducted by the USDA-ARS Environmental Chemistry laboratory (Beltsville, MD). Signed and dated Good Laboratory Practices (GLP), quality assurance, and data confidentiality information were provided. The deviations made to the study protocol and GLP requirements did not effect the conclusions presented in the report.

1. Materials and Methods

1.1. Test Substances

Table 1: List of Analytes Tested.		
Common Name:	glufosinate ammonium	HOE 061517
IUPAC Name:	ammonium-DL-homoalanin-4-yl-(methyl)-phosphinate	3-methylphosphinico-propionic acid
CAS Name:	butonoic acid, (±)-2-amino-4-(hydroxymethylphosphinyl)-, monoammonium salt	3- (hydroxymethylphosphinyl)-propionic acid
CAS Number:	77182-82-2	15090-23-0
Company Name:	HOE 039866	HOE 061517
Other Synonyms:	AE F039866, GA	AE F061517, MP-propionic acid
Structure:		

1.2. Methods

Aqueous NH_3OH (0.015 M) solutions of HOE 039866 and HOE 061517 were prepared and served as the fortification solution. Blueberry control samples were fortified with either the HOE 039866 solution or the HOE 061517 solution to yield a 1.00 ppm concentration and were placed in frozen storage (<-20 C). The samples were stored for 615 (HOE 039866) or 593 (HOE 060517) days and extracted along with control and freshly fortified samples using a modified version of Hoechst-Roussel-Agri-Vet Company Method HRAV-5A. The extracts were analyzed 78 (HOE 039886) or 71 (HOE 061517) days after extraction (stored at <-20 C). The method involves extraction with water, anion exchange, derivatization, silica gel column clean-up, and quantitation via gas chromatography with flame photometric detection. The dervatization step calls for the use of trimethylorthoacetate which esterifies the phosphinic and carboxylic acid functional groups of glufosinate and HOE 061517 and also acetylates the basic amino group of glufosinate. The petitioner reported a limit of quantitation (LOQ) of 0.05 ppm and a limit of detection (LOD) of 0.02 ppm for glufosinate ammonium and a LOQ of 0.03 and a LOD of 0.01 for HOE 061517. Residues in/on controls were <0.02 ppm. The method was adequately validated for data collection purposes.

2. Results

Table 2: Storage Stability of HOE 039866 and HOE 061517 in Blueberry

Commodity	Analyte	Spike Level (ppm)	Storage Period (days)	Storage Temp. (C)	Freshly Fortified % Recovery[1]	Apparent % Recovery	Corrected % Recovery[2]
Blueberry	HOE 039866	1.00	615 - homogenate 78 - extract	<-20	100, 97 avg = 98	93, 94, 96	95, 96, 98
	HOE 061517	1.00	593 - homogenate 71 - extract	<-20	102, 99 avg = 100	73, 72, 72	73, 72, 72

[1] fortified at 0.05 ppm
[2] corrected % recovery = apparent % recovery ÷ average concurrent % recovery

3. Discussion

Control blueberry samples were fortified with HOE 039866 and HOE 061517 at 1.00 ppm and placed in frozen storage (<-20 C). The samples were extracted after 615 (HOE 039866) or 593 (HOE 061517) days of storage and the resulting extracts were analyzed 78 (HOE 039866) or 71 (HOE 061517) days after extraction (extract was stored at <-20 C). The resulting percent recoveries for HOE 039866 (95, 96, 98) and HOE 061517 (73, 72, 72) were acceptable.

4. Deficiencies

No data gaps were identified.

RDI: RAB1 Chemists (20-Jun-2002)
T. Bloem:806R:CM#2:(703)-605-0217:7509C

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050758

Chemical:	**Butanoic acid, 2-amino-4-(hydroxy-methyl**
PC Code:	**128850**
HED File Code	**11000 Chemistry Reviews**
Memo Date:	**06/20/2002**
File ID:	**DPD271110; DPD271223; DPD282757; DPD283373**
Accession Number:	**412-03-0016**

HED Records Reference Center
09/30/2002